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Goat Science

Edited by Sándor Kukovics

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Notice

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Preface

When discussing animal species, it is fundamental to consider the quantity and size of the usable products that come from them. Domestication of the goat began at the border of the Neolithic Period and the Stone Age, about 10,000 years ago. The goat has become a co-animal in the past few years but is mainly kept for its meat, milk and hair, as well as fur production. Accordingly, we use a wide variety of "products". Goats are bred in large numbers of varieties and breeds, and are kept in a wide range of environmental conditions on Earth. Different breeds are also crossed to reach specific goals.

The number of goats kept in the world is slightly smaller than that of sheep (about 1.2 billion heads), but still, more than 1 billion heads offer meat and milk for almost 3 billion people. According to the data of FAOSTAT (2018), 1,002,810,368 heads of goats were kept in the world in 2016, from which more than half were produced in *Asia* (55.45% – 556,019,726 heads). *Africa* was ranked in second place (38.66% – 387,667,193 heads), followed by the *Americas* (3.78% – 37,866,521 heads), *Europe* (1.69% – 16,965,650 heads) and *Oceania* (0.34% – 4,291,278 heads).

China was the biggest (14.87% – 149,091,143 heads) goat-keeper country (as in the case of sheep) in 2016, and the following countries were also part of the top 10 goat-keeper countries: *India* (13.35% – 133,874,637 heads), *Nigeria* (7.37% – 73,879,561 heads), *Pakistan* (7.01% – 70,300,000 heads), *Bangladesh* (5.59% – 56,083,240 heads), *Sudan* (3.12% – 31,325,105 heads), *Ethiopia* (3.01% – 30,200,226 heads), *Kenya* (2.67% – 26,745,916 heads), *Mongolia* (2.55% – 25,574,861 heads) and *Mali* (2.21% – 22,141,497 heads).

Research and development carried out so far and summarised under “goat science” has an enormous number of fields covering large or small results, some of which have been selected for this book. They concern a wide range of knowledge and provide good summaries of results reached in different fields of goat science.

Genetics

The genetic characteristics of goats determine the reproductive and production characteristics of the individuals and varieties, the quality of the products (milk and meat composition, hair length and fines, etc.), as well as the environment in which varieties can be successfully bred. Several publications have already reported on the inheritability and repeatability of each quality trait. Recently, research has increasingly focused on the appearance of certain attributes of molecular, DNA and gene levels, and their level of applicability.

In the first chapter the utility of cross-reactive regents to understand the molecular genetics and genome biology of the goat and the importance of dietary modulators as avenues for immune modulation and maintenance of homeostasis in the goat are the targets of study.

Several kinds of treatment (such as added probiotics, mushroom extracts, etc.) are studied and their effects are summarised in this chapter.

In South Africa, many goat breeds are bred for hair, meat and milk production. The genetic development of imported and indigenous varieties has been going on for decades, but unfortunately the development of breeding stock is historically limited. The limited degree of individual marking and production control and the participation in development programmes, except angora goats, resulted in poor genetic development.

Molecular genetic research has opened up a new opportunity to define certain properties and to preserve the genetic resources represented by these goat varieties. The results of this research are summarised in the second chapter.

Nutrition

It is well known that goats are mainly "browsers", preferring the leaves of trees, bushes and shrubs instead of grazing pastures. In general, if they are allowed to follow their genetic determinations, 80% of goats feed from browsing and only 20% feed from grazing. This mainly happens in extensive goat farming, but in intensive dairy goat production systems, goat nutrition is very similar to that of dairy cattle to reach as high a milk production as possible – in addition to the higher reproduction characteristics.

Based on genetic determinations a question arises: do goats have different passage rates compared to other ruminants? Several factors affect rumen fill levels and the rates of passage through the rumen. These are the animal species and feeding types, stage of reproductive cycle and physiological state, ambient temperature, level of nutrition and feed intake, forage-to-concentrate ratio in the diet, practical size and functional gravity. Taking all these effects into consideration the authors of the third chapter state that despite several differences, goats are not different from other ruminants with respect to passage rate because it is largely due to dietary quality.

Many kinds of mineral deficiency can occur in goats depending on the pastures they graze and the feed they are supplied with. Among others, iodine deficiency could cause several problems in goats, and its symptoms are recognised rather easily. These symptoms and possible and necessary treatments are summarised in the fourth chapter.

Reproduction

The reproduction of goats is quite well known, and most goat keepers are well versed in the details. Goats kept in traditional extensive systems have one oestrus season and one kidding season during the year, which is simple to handle compared to those animals being kept under intensive indoor systems. To be able to modify the reproduction traits of goats it is useful to summarise the most important details from folliculogenesis via physiology and endocrinology of the oestrual cycle, the methods of oestrus synchronisation, artificial insemination and use of the male effect in oestrus induction. An overview of this knowledge can be read in the fifth chapter.

There are many effects on the reproductive performance of male animals. Among others, insulin-like factor 3 (INSL3) is essential for testis descent during foetal development, and has been implicated in testicular and sperm functions in adult males. In the sixth chapter, up-to-date knowledge of the development and impact of these factors has been summarised.

To meet the increasing demands for goat's milk and meat products, production and reproduction efficiency has to be improved. The need for these products continues throughout the world, and in the traditional system of production may offer only seasonal supplies. The utilisation of new reproduction methods and reproductive biotechnological technics is more and more important in the case of females but is also important in male goats too. In the seventh chapter the main knowledge regarding oestrus synchronisation and manipulation, artificial insemination, and the use of the male effect are summarised.

Milk production

Goat's milk is not as popular as cow's milk among consumers; however, goat's milk has several benefits and nutritional values, which are missing from cow's milk. Over the last couple of decades the rearing of dairy goats became an economic activity, which is rather concentrated in certain regions and countries of the world. In addition, goat's milk and milk products have become rather significant parts of foods for more than 1 billion people.

According to the FAOSTAT (2018) data, 15,262,116 tons of goat's milk was produced in the world in 2016, from which *Asia* had the biggest share (55.70% – 8,043,749 tons), followed by *Africa* (25.74% – 3,928,719 tons), *Europe* (16.63% – 2,537,787 tons), the *Americas* (4.93% – 751,823 tons) and *Oceania* (0.00 – 39 tons).

Studying the 10 biggest goat's milk-producing countries (FAOSTAT, 2018), the following order was determined for the year 2016: *India* (24.69% – 3,767,866 tons), *Sudan* (7.24% – 1,104,620 tons), *Bangladesh* (6.89% – 1,051,493 tons), *Pakistan* (5,40% – 824,098 tons), *France* (3.95% – 603,040 tons), *South Sudan* (3.06% – 466,672 tons), *Spain* (2.69% – 410,977 tons), *Greece* (2.52% – 384,903 tons), *Somalia* (2.47% – 377,733 tons) and *Indonesia* (2.46% – 375,453 tons).

In 2016 (FAOSTAT, 2018) there were 202,004,520 milking goats registered, from which the biggest herd was milked in *Asia* (52.11% – 105,619,554 heads), followed by *Africa* (39.60% – 80,272,622 heads), *Europe* (4.32% – 8,748,735 heads), the *Americas* (3.97% – 8,047,754 heads) and *Oceania* (0.00% – 1351 heads).

Concerning the order of the countries having milking goats (FAOSTAT, 2018) the following order could be observed in the year 2016: *India* (14.40% – 29,180,066 heads), *Bangladesh* (14.01% – 28,400,285 heads), *Sudan* (9.31% – 18,878,691 heads), *Mali* (8.19% – 16,606,239 heads), *Pakistan* (4.05% – 8,212,610 heads), *Indonesia* (3.79% – 7,686,690 heads), *South Sudan* (3.43% – 6,943,044 heads), *Somalia* (3.12% – 6,327,494 heads), *Niger* (2.72% – 5,514,053 heads) and *Turkey* (2.31% – 4,687,028 heads).

The composition of the goat's milk produced is the target of various scientific studies.

The protein content of goat's milk is one of the most important values in its utilisation. The main groups of proteins are the caseins, whey proteins, and milk fat globule membranes. Determination of these components in detail and the possible experimental and industrial methods, as well as the equipment used, is the target of much research and development work.

One of the important animal health problems in milk production is mastitis (udder inflammation) and in relation to this are the somatic cell counts of various milk items. Mastitis could have a deep impact on protein composition and the processability of the milk. The progress of proteomics applications and an overview of developments in the field of goat's milk are provided in the eighth chapter of this book.

So far, many scientists have dealt with the secret of the goat mammary gland and tried to model its establishment, characterisation and the application of primary goat mammary cell culture to understand the details of its functioning. In the ninth chapter the authors provide a good summary of the knowledge in this field.

The short- and medium-length fatty acid content gives a specific taste and odour to goat's milk and milk products. Medium fatty acid synthesis in the goat mammary gland still has a number of unknown details, which are summarised and evaluated in the tenth chapter.

Milk and health

There are many publications reporting the health benefits of goat's milk, and a number of them give new reasons why the consumption of goat's milk is highly advantageous. According to the statements of many scientific publications and promotional announcements, the advantages of goat's milk could be summarised as follows. The frequent and systematic consumption of goat's milk helps to build strong bones, it has significant anti-inflammatory properties, it increases nutrient uptake efficiency, it can be used as a metabolism booster and at the same time can boost the immune system as well as protect the heart. It can help one's growth and development at various stages of life, and in several cases could give a "helping hand" in weight loss. In addition, goat keeping possesses serious environmental protection qualities by preventing bush fires and exterminating unwanted weeds and plants simply through grazing.

However, goats produce red meat, but this meat is very lean, and because of its composition, vitamins, proteins, minerals, as well as fat content, goat's meat could be considered an essential part of human nutrition.

Keeping these positive effects at the forefront, nutritional and health profiles of goat products are presented in the eleventh chapter where the focus is on goat's milk. In the twelfth chapter the relationships between feeding systems and various breeds as well as the bioactive compounds of goat's milk and cheeses are summarised and evaluated.

Goat's meat

World goat's meat production consisted of 5,621,133 tons in 2016 according to FAOSTAT (2018) data. Among the continents, *Asia* (73.18% – 4,113,646 tons) dominated goat's meat production. *Africa* took the second stage (22.13% – 1,244,109 tons) with slightly more than one-third of the *Asian* data. The *Americas* were in third place (2.26% – 127,041 tons). *Europe* was in fourth place with limited data (1.76% – 98,934 tons) and *Oceania* was last (0.67% – 37,603 tons).

Concerning the 10 biggest goat's meat-producing countries, *China*'s leadership was obvious (40.33% – 2,266,998 tons). *India* took second place (8.99% – 505,371 tons) but provides less than one-quarter of goat's meat in compare with *China*. The following countries were also in the top 10: *Pakistan* (5.37% – 301,589 tons), *Nigeria* (4.33% – 243,230 tons), *Bangladesh* (3.71% – 208,719 tons), *Sudan* (1.98% – 111,532 tons), *Mali* (1.70% – 95,415 tons), *Islamic Republic of Iran* (1.48% – 82,958 tons), *Myanmar* (1.41% – 79,418 tons), *Mongolia* (1.37% – 76,903 tons) and *Yemen* took eleventh place with 65,872 tons.

Many scientists have declared that goats are resilient and prolific small ruminants with the ability to adapt to a wide range of ecological circumstances. Comparing the possibilities and values of this kind of meat, production and consumption are still not as great as other meats.

Goat's meat serves as a major source of meat in less developed countries, but it is less popular in developed countries. This situation is forecast to change because of the health benefits of consuming lean meat with limited fat and cholesterol contents.

Besides the huge differences in consumption trends among less developed and western countries, many factors modify the value of goat's meat and the marketability of the carcass. These are not only the breed, type, age, weight of slaughter and gender of slaughtered animals, but also the production system, nutrition, type of suckling, as well as ageing of the meat. These effects are evaluated in detail in the thirteenth chapter of this book.

Animal health

The factors that affect parasitic infection in livestock may be divided into three groups, which co-exist and overlap each other. Members of first group are highly dependent on the animal itself: genetics, ageing, reproduction phases (like pregnancy, etc.), immune status, nutrition status and hypobiosis. The second group is dominantly based on farm management: grazing, co-grazing, alternate grazing, housing system and drenching, as well as handling the animals. The third group of factors originated from the environment: temperature, moisture, sunlight, grass composition and the varieties of worm species. At the same time, the effects of all these are highly dependent on gut immunity and various kinds of therapeutics, as well as resistance of various kinds of worms against the different agents of the drugs used. Several parts of this matrix are evaluated in the fourteenth chapter, including the various kinds of plants that could be used as natural treatments against internal parasites.

Because the use of huge amounts of various kinds of medicines was noticed a new approach to reducing the quantity of medicines – especially antibiotics – has become more and more important. Homeopathy, meaning that one should use different agent molecules in various levels of dilutions, has caught the attention of several scientific teams. However, many scientists doubt the real benefits of highly diluted molecule agents, and a wide range of information is summarised in the fifteenth chapter.

Heat stress

However, the goat is one of the most adaptable animal species and its production level is highly dependent on environmental circumstances. Along with global warming, heat stress is becoming more and more important even in the case of goats. There are serious physiological and metabolic consequences of heat stress, which have strong impacts on milk and meat production and even reproduction characteristics, as well asmilk composition. It is necessary to have a more exact understanding of the genetic background of heat tolerance and the complexity of an animal's reaction to thermal stress. The most up-to-date information is summarised and evaluated in the sixteenth chapter of this book.

Production systems

Goats were traditionally kept mainly in extensive production systems, so-called traditional grazing. In spite of this and especially as a reason to increase the demand for milk and milk products, traditional goat keeping has been converted to intensive indoor systems; however, several kinds of semi-intensive systems are also used. The intensification of goat's milk production started mainly in Europe (especially in France, Spain, the Netherland and Greece) and then started to spread to other parts of the world. This process became so successful that intensive dairy goat breeds (Alpine, Saanen, Toggenburg, Nubian, etc.) and the production system (including housing, feeding, milking, and reproduction) were needed for their

expected level of production and can now be found in almost every goat-keeper country; however, these breeds are mainly dominant in Europe. This is the main reason why goat's milk production is much higher in Europe – considering the number of goats kept – than in other continents of the world.

The relationship between the environment and production systems used and the products made is summarised in the seventeenth chapter of this book. There are several advantages and some disadvantages to the animals, the environment and especially to the farmers.

In Jordan, goats are kept mainly for meat production and less for milk, skin and fibre, and the production system is based on the utilisation of low-quality roughage. The eighteenth chapter shows how the extensive production system is being gradually shifted toward a more intensive way of production using feeds grown on arable lands.

The changing production system and the traditional way of goat keeping in Lebanon are summarised in the nineteenth chapter of this book. There is a rising demand for traditional goat's milk products, but this demand could not be served from the goat's milk produced using traditional systems.

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Genetics

Chapter 1

Molecular Genetics and Genome Biology of Goats

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Emmanuel Asiamah and Mulumebet Worku

Additional information is available at the end of the chapter

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Abstract

Information on goat genome has led to a better understanding of the genetics of goats, its response to infection and the underlying immune response mechanism. Natural product-based therapeutic can therefore be utilized to target genes important for goat immunity. In this chapter, we have summarized the effect of diet and dietary supplements as immune modulators in goats. These modulators affect the expression of genes and secreted proteins associated with innate and adaptive immune response and homeostasis. Probiotics, mushroom extracts, plant polyphenol extracts, Sericea lespedeza (SL) and cowpea diet affect key molecular pathways including Toll-like receptor (TLR) pathway, Wnt signaling pathway and cytokine-mediated signaling pathway. Results from various studies reviewed in this chapter suggest that utilization of dietary immunomodulators has beneficial effects on goat health and production.

Keywords: blood, gene expression, transcription, translation, modulation, innate immunity, homeostasis

1. Introduction

The domestic goat (*Capra hircus*) is an important farm and companion animal species. They are descendants of the bezoar (*Capra aegagrus*) goat. The world goat population has been on the increase during the last three decades and is estimated to be 1 billion with the global genetic diversity characterized by more than 590 breeds [1]. Breeds have various advantageous characteristics such as adaptation to harsh environmental conditions, capacity to convert poor quality fibrous feedstuff into animal proteins and resistance to diseases. Known as 'the poor man's cow,' goats are primarily reared for meat production, but different breeds of goat are often used as a source of milk and wool. Goats are also used for carrying small loads and for land management and kept as pets.

In the United States, production of small ruminants is a growing industry as a result of high demand for grass-fed or organically produced livestock [2]. Compared to all other livestock enterprise, goat production requires minimal capital input and low cost of breeding stock [3]. Globally, healthy goats are crucial for the long-term success of the goat industry. Production is negatively challenged by factors such as feed toxins, respiratory diseases (pneumonia) and other infectious diseases. Goats are also susceptible to viral diseases (foot and mouth diseases) and bacterial diseases (mastitis). Gastrointestinal (GI) nematode infection is considered the most important limiting factor in goat production systems around the world and results in huge economic losses to producers.

However, resistance to current drugs and lack of interest in developing new drugs by companies pose a challenge for sustainable ruminant production. The widely used approach for the treatment of infection by parasite is drug treatment. Measures used to reduce parasite infection include the reduction of stock density and the maximization of pasture to reduce parasite numbers. Plant-based anthelmintic is also being explored for use in the elimination of gastrointestinal parasites including extracts such as: garlic, neem, wormwood, tobacco, cowpea [4–6] and Sericea lespedeza [7]. Several other alternatives that have been proposed include the use of nonchemical additives such as probiotics [8] and prebiotics, the improved production practices and the use of genetics-based breeding schemes. Improvement in animal nutrition can also impact the immune response including gene response, protein synthesis, modification and degradation, metabolism, signal transduction and cellular proliferation [9]. The understanding of protective mechanisms regarding the initial steps of the host's response to pest or parasite-derived molecules that can correlate with resistance or susceptibility to pathogens needs to be explored. This understanding will aid the design of immunomodulatory strategies to induce a change in the magnitude of immune or nonimmune responses.

2. The goat genome

In the last decade, molecular genetics has led to the discovery of individual genes or candidate genes with substantial effects on our understanding of homeostasis and immunity. The goat genome has been sequenced, and raw sequences have been deposited in NCBI (GenBank, CapAeg_1.0 (GCA_000978405.1) under the accession no. SRA184825. Utilizing information on the goat genome enables a better understanding of the genetics of the goat and how it responses to infection and disease and fights it naturally. The sequencing of the goat genome has led to increased understanding of the genetics underlying immune response mechanism [10]. Genes significant for goat immunity can be targeted by using natural products and plant-based therapeutics for improving goat health. This eliminates the need for chemical treatment, buildup of antibiotic resistance and food insecurity concerns among goat consumers.

2.1. Innate immune system

The main function of the immune system is to distinguish between own cells and tissues from external cells and tissues in order to protect against infestation. The immune system has

various mechanisms to eliminate or withstand the impact of external agents. The animal's immune system is composed of two related functional elements: the innate immune system and the adaptive immune system [11]. Both function in coordination to protect against invading microorganisms [12]. Innate immunity is the first line of defense against organisms; it acts in a nonspecific way through anatomical barriers (skin, mucus membrane), secretions, cells and other elements. The adaptive immune system is the second line of defense which responds slower than the innate immunity system. Innate immune defense plays a key role in affording protection [13]. Unlike the innate immune system, the adaptive immune system has the ability to 'memorize' infectious agents allowing the adaptive immune system to serve as a rapid response system if pathological agents are encountered again [12]. The innate immune system consists of natural killer cells, T-cell and B-cell, basophils, eosinophils, monocytes, macrophages and polymorphonuclear neutrophils. These cells are called white blood cells or leukocytes and are also divided into two groups based on their morphology: granulocytes and agranulocytes. Granulocytes include eosinophils, neutrophils and basophils, and agranulocytes are lymphocytes (T and B cells) and macrophages [14]. A differential white blood cell count is an important tool used to provide clinical diagnosis and for monitoring of disease and blood disorders [15]. This system quantifies and differentiates white blood cells at one particular time giving an insight into infection and checking whether treatments are working [15].

2.2. Toll-like receptors

Animals live in a wide variety of microbe-rich environments, and hence, it is crucial to have a sensitive innate defense mechanism which relies in part by recognizing conserved molecules that are unique to some classes of potential pathogens [16]. It is very important to understand the innate immunity against microbial components and its critical role in host defense against infection. Toll-like receptors (TLRs) have been shown to participate in the recognition of pathogens by the innate immune system.

Toll-like receptors (TLRs) are a highly conserved group of proteins that have been identified in mammals [17]. The TLR family consists of 10 receptors: TLR-1-10, which are very important in the identification of microbes [18]. The coding regions within the goats, TLR-1-10 genes, have been sequenced and found to be conserved and highly similar in nucleotide composition [10]. With the discovery of Toll-like receptors (TLRs), studies have shown that pathogen recognition by the innate immune system is broadly specific, which relies on germline-encoded pattern-recognition receptors (PRRs) to detect relatively conserved components of pathogens referred to as pathogen-associated molecular patterns (PAMPs) [19]. The PAMPs recognized by TLRs include lipids, lipoproteins, proteins and nucleic acids derived from a wide range of microbes such as bacteria, viruses, parasites and fungi [20] which initiates a complex signaling cascade to activate a wide variety of transcription factors and inflammatory cytokines [18].

2.3. Cytokines

Cytokines are small proteins that transmit information from one cell to another. The analysis of cytokines secreted by immune cells in response to infectious agents is crucial to understand pathogenesis and immunity. Most cells in the body produce cytokines during inflammatory

processes which represent a large series of regulatory proteins of the immunologic system. Many cytokines are referred to as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Two general patterns of cytokine secretion by such cells have been described. In the Th1 response, cytokines initiate cell-mediated reactions defined as the activation of macrophages to combat infectious pathogens by releasing IL-1, IL-2, IL-8, and IL-12 to activate inflammation [21]. In the Th2 response, T-helper cells activate B-cells; interleukins IL-4, IL-5, IL-6, IL-10 and IL-13 are released to counter infectious agents caused by extracellular organisms [21]. Studies have shown that the release of cytokines is essential for host survival from infection and is also required for tissue repair.

2.4. Wingless pathway

The Wingless (Wnt) signaling pathway is a conserved pathway in mammals. It involves Wnts, which are secreted glycoproteins that are associated with the Wnt-1 and Wingless gene products of Drosophila [22]. Activation of Wnt signaling happens when Wnt ligands binds to Frizzled receptors together with other receptors lipoprotein receptor-related protein (LRP) 5 and 6 [23–25]. About 19 Wnt ligands and 10 Frizzle receptors have been identified in metazoan mammals. The receptor-ligand interaction leads to downstream signal regulation which is categorized into two: canonical (Wnt/β-catenin) and noncanonical pathways. The former is dependent on β-catenin, but the latter is not. The noncanonical pathway is further subdivided into the planar cell polarity and the Wnt/Ca^{2+} pathways. The Wnt signaling pathway function in cellular processes includes cell proliferation, cell differentiation, cell migration, cell polarity and cell fate determination and has recently been implicated in stem cell renewal [26]. Wnt signaling has also been associated with various biological processes including adipogenesis, myogenesis, embryogenesis and meat quality. In addition, Wnt signaling has been associated with innate immune and inflammation responses via a cross talk with the TLR and NF-κB pathways [27, 28]. Therefore, a defective or deregulated Wnt signaling has detrimental effect on developing embryo (birth defects) and also affects a number of pathological disease conditions.

3. Methodologies for goat studies

3.1. Evaluation of phenotypic parameters

Various phenotypic characteristics are measured in goats following treatment with supplements or feeds in a study. Usually, body weight, body condition score and FAMACHA score are recorded periodically as a measure of effect on growth and health. Body weights are taken before morning feeding using a portable scale [8]. Body condition is scored on a scale of 1–5 by physical examination of the goat's body as described by Villaquiran et al. [29]. Blood samples collected aseptically are evaluated for packed cell volume (PCV) and white blood differential cell counts. PCV is widely used as an indicator trait for anemia. White blood differential counts are measured using the procedure described by Schalm et al. [30]. Fecal samples are collected directly from the rectum and evaluated for the number of parasite egg counts.

More specifically, the number of strongyle eggs and coccidia oocytes is measured using the modified McMaster method [31]. The fecal eggs counted are multiplied by 50, and resulting total is expressed as eggs per gram (epg) of fecal sample per animal [14].

3.2. Molecular techniques

The molecular effects of immunomodulators have been evaluated in goats at the gene transcription and protein levels using different techniques including real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).

3.3. Real-time PCR

Quantitative real-time PCR is used to measure messenger RNA (MRNA) levels [77]. For real-time PCR analysis, total RNA is isolated from whole blood cell pellets using Trizol method or QuickRNA MiniPrep Kit (Zymo Research) as per manufacturer's procedure. The concentration and purity of the RNA are checked on NanoDrop Spectrophotometer (ND-1000; Thermo Fisher). Mostly, a pure RNA typically yields a 260/280 ratio of ~2.0 and this is considered ideal. A 260/280 ratio below 2.0 suggests protein contamination [82]. In addition, the integrity of the RNA (RNA integrity number (RIN)) can be measured with a bioanalyzer, and a RIN <7.0 indicates a good RNA. Since RNA is not stable, it is converted into more stable complimentary DNA (cDNA) using cDNA conversion kits containing oligo (DT) and random primers, reverse transcriptase, and other needed reagents as specified in the manufacturer's manual. Real-time PCR is performed with reaction mixture comprising of cDNA template, primers and SYBR Green [78]. Housing-keeping genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin (*ACTB*), ribosomal protein L32 (RPL32), TATA sequence binding protein (TBP) and cyclophilin are used as internal controls for normalization of the RT-PCR data obtained [32]. The RT-PCR data are analyzed by calculating fold change in the expression of the specific genes tested using statistical approaches including the comparative C_T (also known as $2^{-\Delta\Delta C}{}_T$ or Livak's method).

3.3.1. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a molecular assay used for analytical detection and quantification of specific antigens or antibodies in a given sample. It uses the concept of an antigen binding to its specific antibody which enables detection of antigens such as proteins, peptides and antibodies [33]. With ELISA, goat serum or plasma is evaluated for the levels of immune response and inflammation biomarkers such as cytokines, prostaglandin and immunoglobulins. Cytokines measured in goat serum following dietary supplementation include TNFα, IL-1β, IL-8, GCSF, GMCSF, Rantes and IFNγ [7, 8, 34]. The levels of secreted prostaglandin E, an eicosanoid and also an inflammation mediator have also been measured in goat serum and plasma with ELISA [34–36].

3.4. Effect of pathogen-associated molecular patterns

Goats rely on pasture as their main source of feed. Studies have been done to elucidate the effects of different PAMPs, microbe-associated molecular pattern (MAMP) and plant polyphenol

metabolite in animal feed on goat health. Pathogen-associated molecular pattern evaluated in goats includes the following: probiotics, mushroom, plant polyphenols, cowpea, lipopolysaccharide (LPS), peptidoglycan, nystatin and Sericea lespedeza (**Table 1**).

3.4.1. Probiotics

Probiotics has been studied and considered as health beneficial microorganism which plays a role in maintaining homeostasis. Previous studies have shown the use of probiotics to modulate gastrointestinal health. Liong [37] reported the resistance to infectious diseases in the gastrointestinal tract as a result of probiotics. Probiotics as a supplement in animal feed has shown to have a beneficial effect on milk yield, fat and protein content [38]. Ekwemalor et al. [39] reported the release of proinflammatory cytokines in goats orally drenched with probiotics (*Coriolus versicolor* [CV]). Previous study conducted by our research team looked at the molecular impact of probiotic administration on physical health parameters and activation of genes involved in homeostasis and immunity in goat blood. We reported that genes associated with innate and adaptive immunity were modulated as a result of probiotics treatment. Genes that were expressed are associated with the host response to bacteria, virus, T-cell activation, cytokines and inflammatory response. **Table 2** shows genes modulated as a result of probiotic modulation.

Modulator (s)	Sample type (s)	Cytokines	Innate immune response	Reference
Probiotics	Whole blood, serum	IL2, IL5, IL10, IL8, IL18	TLR4, TLR6, TLR7, TLR9	[80, 81]
Plant extract	Whole blood	—	TLR2	[54]
Cowpea	Whole blood, serum, plasma	TNFα, IL1α, ILβ, IL8 *IL10RA*, IL15, IP10, G-CSF, Rantes and IFNγ	TLR2	[34, 69]
Sericea lespedeza	Whole blood, serum	TNF-α, IFNr, GCSF, GMCSF, IL-1α, IP-10	TLR2 and TLR4	[7]
Mushroom	Neutrophils, whole blood, serum	IFNr, Rantes and granulocyte colony stimulating factor (GCSF). granulocyte macrophage colony-stimulating factor (GM-CSF)	TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10	[8]
Lipopolysaccharide	Mammary epithelial cells, whole blood, blood leukocytes	IL1B, CCL3 and IL8, *CCL2, CXCL6, IL6, CXCL8*	*PTGS2, IFIT3, MYD88, NFKB1, and TLR4*	[54, 83]
Peptidoglycan	Whole blood	—	TLR2	[54]
Lipoteichoic acid	Mammary epithelial cells	*CXCL6, CXCL8, CCL5*	*PTGS2, IFIT3, NFKB1, TLR4, and TOLLIP*	[84]

Table 1. List of immunomodulators tested on goats.

Category	Genes	Reference
Pattern recognition receptors (PPRs)	DDX58, NLRP3, NOD1, NOD2, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9	[8]
Cytokines	CCL2, CCL5, CSF2, CXCL10, IFNA1, IFNB1, IL18, 1L1A, IL1B, IL2, CXCL8, TNF	[8]
Innate immunity genes	APCS, C3, CASP1, CD14, CD4, CD40, CD40LG, CD8A, CRP, HLA-A, HLA-E, IL1R1, IRAK1, IRF3, IRF7, ITGAM, LY96, LTZ, MAPK1, MAPK8, MBL2, MPO, MX1, MYD88, NFKB1, NFKB1A, STAT1, TICAM1, TRAF6	[8]
Th1 markers & immune response	CCR5, CD80, CXCR3, IFNG, IL18, IL23A, SLC11A1, STAT4, TBX21, TLR4, TLR6	[8]
Th17 markers	CCR6, IL17A, RORC, STAT3	[8]
T-cell activation	CD80, CD86, ICAM1, IFNG, IL23A, 1 L6, SLC11A1	[8]
Treg markers	CCR4, CCR8, FOXP3, IL10	[8]
Adaptive immunity genes	CD40, CD40LG, CD8A, CRP, FASLG, HLA-A, IFNARI, IL1B, IL1R1, IRF3, IRF7, ITGAM, JAK2, MAPK8, MBL2, MX1, NFKB1, RAG1, STAT1	[8]
Inflammatory response	APCS, C3, CCL5, CRP, FOXP3, IL1A, IL1B, IL4, IL6, MBL2, STAT3, TNF	[8]
Defense response to bacteria	IFNB1, IFNG, IL23A, IL6, LYZ, MBL2, MYD88, NOD1, NOD2, SLC11A1, TLR1, TLR3, TLR4, TLR6, TLR9, TNF	[8]
Defense response to viruses	CD4, CD40, CD86, CD8A, CXCL10, DDX58, HLA-A, IFNARI, IFNBI, IL23A, IL6, NLRP3, TICAM1, TLR3, TLR7, TLR8, TYK2	[8]

Table 2. List of genes associated with innate and adaptive immunity.

Researchers have reported effects of probiotics in goats of which most effects have been attributed to an increase in the innate immune system and others in the acquired immune response. Leeber et al. [40] reported that probiotics have the properties to modulate host immune system through different signaling pathways of innate immune cells. The innate immune system functions by initiating a response to microorganisms or their components via pattern recognition receptors such as nucleotide-binding oligomerization domain-like receptors or TLR [41]. Previous studies conducted by Worku and Morris [42] and Worku et al. [7] have shown the expression of TLRs in whole blood.

When ligands bind to TLRs, they trigger at least two most important cell signaling pathways. One of the pathways involves MyD88, an adaptor protein which is shared by most TLRs. When this pathway is triggered, it leads to the activation of the transcription factor NF-κB which then results in the release of proinflammatory cytokines [10, 43, 44]. Ekwemalor et al. [8] reported that probiotics modulated the expression of genes in myeloid differentiation antigen 88 (MYD88)-dependent or MYD88-independent system, TLR-mediated signaling induction pathway, nuclear factor κB (NF-κB), cytokine-mediated signaling pathways and Wnt signaling pathway. **Table 3** shows the different genes that were expressed in the Wnt signaling pathway involved in canonical Wnt signaling, planar cell polarity, negative regulation, calcium signaling, cell growth and proliferation as a result of probiotics.

3.4.2. Mushrooms (Coriolus versicolor)

Mushrooms have been studied and are known for their nutritional and medicinal properties. They contain bioactive compounds which are of medicinal importance. There are several types of mushroom of which *Coriolus versicolor* (CV) is one of the studied types of mushroom because of its medicinal properties. They contain active ingredients such as polysaccharide krestin (PSK) and polysaccharide peptide (PSP) [45]. Eliza et al. [46] reported that extracts of CV have the potential of boosting suppressed immune function, extending the survival rate and improving quality of life. They exert their therapeutic effects by modulating the host's immune response. Zhou et al. [47] demonstrated their effect in stimulating the immune system and inhibition of cancer growth. Lull et al. [48] also reported their effect in activating T and B lymphocytes, macrophages, natural killer cells, and lymphocyte-activated killer cells, as well as promoting the production of antibodies and various cytokines. Results from research team showed that mushroom extracts of CV modulated the expression of 10 TLR in neutrophils and modulation of innate immunity through differential regulation of the secretion of serum proteins including cytokines and prostaglandin E2 to impact goat health [8].

3.4.3. Lipopolysaccharide, peptidoglycan and nystatin

Bacteria produce molecules such as lipopolysaccharide (LPS), lipoproteins, peptidoglycan and lipoteichoic acids (LTAs), and this serves as specific molecular signatures for different classes of bacteria [49]. Lipopolysaccharides (LPSs), also known as lipoglycans, are the main surface membrane of Gram-negative bacteria. LPSs comprise poly- or oligosaccharide region and lipid A, which is the main immunostimulatory part of LPS [50]. Lipopolysaccharide is recognized by TLR4 assisted by CD14 proteins [49].

Category	Modulator	Genes	Reference
Canonical WNT signaling	Probiotics	APC, AXIN2, CSNK1A1, DVL2, FZDI, FZD7, FZD8, GSK3A, GSK3B, LEF1, LRP5, NKD1, PORCN, RUVBL1, SFRP4, TCF7, TCF7L1, WIF1, WNT1, WNT2, WNT3A, WNT7B, WNT8A	[80]
Wnt signaling target genes	Probiotics	CCND2, WISP1	[80]
Planar cell polarity	Probiotics	DAAM1, MAPK8, VANGL2,	[80]
Proliferation	Probiotics	DAB2	
WNT signaling negative regulation	Probiotics	FBXW4, FBXW11, FRZB	[80]
Cell growth and proliferation	Probiotics	FOXN1, JUN, MMPZ, PPARD	[80],
WNT calcium signaling	Probiotics Nystatin	NFATC1, WNT5B, WNT5A	[35, 80]

Table 3. Differentially expressed genes on the Wnt signaling pathway in response to modulators.

Peptidoglycan and lipoteichoic acids are the major stimulatory components of Gram-negative bacteria and are recognized by TLR2 [51]. Pathogen recognition receptors, such as TLRs, have evolved to recognize these PAMPs and detect invading disease microbes [49].

The linkage between PAMPs, TLR, activation of the prostaglandin pathway and the promotion of Wnt signaling in inflammatory response has been studied [52, 53]. Previous work by Asiamah et al. [54] also indicates that TLR2 and Frizzled receptors are increased in response to bacterial cell wall components (lipopolysaccharide, peptidoglycan). Nystatin is a lipid raft inhibitor derived from the bacterium *Streptomyces noursei*, and in addition, a proinflammatory agent was also found to modulate TLR2 and Frizzled receptor in goat blood. These findings open a window into the innate immune mechanism and inflammatory response mediated through TLRs and Wnt in goats and may provide more understanding about disease resistance as well as aid in drug design and animal selection through breeding programs.

3.4.4. Plant polyphenols

Apart from plants being important feed resource for animal nutrition, they are also a rich source of polyphenol bioactive compounds that have beneficial health effects. Polyphenols (also known as phenolic compounds) are naturally occurring plant metabolites and are an integral part of both human and animal diet [55]. These compounds include flavonoids, tannins, phenolic acid and others [56]. Feed resources containing tannins have been reported to have both beneficial and detrimental effects on grazing animals. Tannin-rich plants have direct antiparasitic activity but might also act indirectly by increasing host resistance. These effects vary depending on the species of plant, parasite and host [57]. The antiparasitic potential of forage legumes (Fabaceae family), including sulla (*Hedysarum coronarium*) [58], sainfoin (*Onobrychis viciifolia*) [59], birdsfoot trefoil (*Lotus corniculatus*) [60], big trefoil (*Lotus pedunculatus)* [61] and Sericea lespedeza (*Lespedeza cuneata)* [62–64], herbs and fodder trees have been evaluated in goats.

The roles of polyphenol extracts from plants in the immune function have been reported on different cell types both *in vitro* and *in vivo* [65]. Polyphenol and other plant extracts demonstrated the ability to induce the release of both proinflammatory and anti-inflammatory cytokines, thus leading to the maintenance of the immune homeostasis in the host [66]. Epigallocatechin-3-gallate, a flavonoid found in green tea, has been shown to inhibit NF-κB activation induced by many proinflammatory stimuli [67]. Recently, there is a great interest in feed polyphenols due to their antioxidant capacity, inflammatory and immunomodulatory properties and their possible beneficial implications on animal health and production [7, 54, 68, 69, 79].

3.4.5. Sericea lespedeza

Sericea lespedeza (*Lespedeza cuneata*) is a leguminous plant with high tannin content. It has been studied extensively for its possible anthelmintic potential especially in small ruminants [60]. A study by Worku et al. [7] demonstrated the impact of Sericea lespedeza (SL) diet on innate immune response mediators in goats. More specifically, Sericea diet increased serum level of proinflammatory cytokines TNF-α, IFNr, GCSF, GMCSF, IL-1α and IP-10 ($P < 0.0002$)

and decreased ($P < 0.0001$) IL-8 and RANTES. In addition, results from gene expression analyses showed increased mRNA transcripts of cell surface receptors TLR2 and TLR4, and the cytokines IL-8, IL-10, IL-2 and INF-γ. Previous work by Asiamah et al. [54] also demonstrated that transcription of TLR2 and Frizzled receptor in goat blood is variably responsive to Sericea lespedeza. In summary, goats respond to plant extracts and may have an effect on the expression of innate immune markers. This may offer an avenue for the exploitation of plant-derived tannins to regulate inflammatory response and enhance goat innate response.

3.4.6. Cowpea

Cowpea (*Vigna unguiculata,* L. Walp) is a highly nutritious legume plant used as human food and feed for animals. It has been utilized as a supplement feed to enhance feed intake and improve productivity in ruminants fed low-quality roughage diets [70, 71]. Cowpea also contains polyphenol compounds including phenol acids, flavonoids and tannins [72]. Polyphenolic extract of cowpea has been shown to have potential impact on ruminant health via antioxidant capacity [68], anti-inflammatory properties [34, 73] and modulating expression of genes associated with immunity and homeostasis [6, 43, 69]. Treatment with cowpea extract downregulated the expression of proinflammatory cytokine TNFα (fold change (FC; treatment/control) = −43.39), IL1α (FC = −6.19), ILβ (FC = −3.62) and IL8 (FC = −1.25). Also, CPE modulated the expression of *IL10RA* (a receptor for IL10, an anti-inflammatory cytokine) and IL15 [73].

A study by Adjei-Fremah et al. [69] demonstrated the impact of cowpea forage grazing, particularly Mississippi Silver variety on growth, internal parasite burden, and markers of immunity in goats. Their study results showed a modulation in cytokines levels, TNF-α, IL-8, and IP10 decreased, whereas an increase in G-CSF, Rantes and IFNγ was observed. The total antioxidants in plasma also increased in the cowpea-grazed goats [34, 73]. Cowpea diet may therefore stimulate innate immune response in goats, and this will help the animals fight against infectious pathogens and diseases. The immunomodulatory potential of cowpea feed may be due to at least in part their polyphenols [68, 74, 75]. Phenolic compounds in animal feeds have antioxidant properties that prevent the damaging effect of free radicals and their metabolic by-products [76] and stimulate an immune response in animals [75].

4. Conclusion

These studies provide an insight into the utility of cross-reactive regents to understand the molecular genetics and genome biology of the goat and importance of dietary modulators as avenues for immune modulation and maintenance of homeostasis in the goat. Treatment with probiotics, mushroom extracts, PAMPS and plant-derived PAMPS resulted in differential expression of genes related to TLR signaling and WNT signaling pathway. Greater insight is provided into goat molecular genetics and genome biology, conserved and novel genes and signaling pathways. Gene expression and modulation has implications for the design and development of innovative therapeutics. Novel goat-specific and conserved gene expression patterns have been identified and provide insight into the utility of genome analysis for the better definition of the mechanism of action of modulators of gene expression in the goat for improved production and welfare.

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Chapter 2

The Development and Genetic Improvement of South African Goats

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Additional information is available at the end of the chapter

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Abstract

South Africa has a thriving goat industry, consisting of fiber, meat and dairy-producing goat breeds. These animals play an important role in terms of food security, socioeconomic welfare and cultural well-being. The South African goat industry is differentiated into a formal, commercial market with niche products such as mohair, chevon and goat's cheeses versus the informal, mainly meat-producing sector serving communal and smallholder farmers. Exotic and locally improved breeds, i.e., Angora, Saanen and Boer goats mainly serve the commercial industries, whereas the unimproved veld goat populations are well adapted in the resource-poor environments. Genetic improvement has historically been limited to the commercial breeds, but poor participation in animal recording and improvement schemes have resulted in slow genetic progress, with the exception of the Angora goat. Molecular research has opened up new possibilities for genetic characterization, preservation and utilization of the unique genetic resources retained by these animals.

Keywords: animal recording, genetic parameters, indigenous breeds, molecular genetics, quantitative selection

1. Introduction

South Africa contributes almost 50% to the Southern African goat population [1] with approximately 5.62 million animals [2] distributed throughout nine provinces. Approximately 2 million of these animals are found in the Eastern Cape Province, almost 1 million in Limpopo Province and just over 700,000 in KwaZulu-Natal Province. The remaining provinces share the remaining 1.8 million animals. The Angora goat population of approximately 640,000 goats is the major contributor to the income generated in the formal goat sector, by supplying

more than 50% of the global mohair clip. The commercial meat goat industry, consisting of the Boer, Savannah and the Kalahari Red breeds makes up 1.3 million goats, with commercial dairy goats being the smallest sector, with approximately 4000 registered dairy goats. The majority (approximately 63%) of South African goats consist of unimproved indigenous veld goats in the noncommercialized agricultural sector and are kept under small-scale conditions.

2. Goat breeds of South Africa

In South Africa, there are seven goat breeds that are officially recognized by the Animal Improvement Act No. 62 of 1998, which includes the Angora goat for mohair production, three meat types namely the South African (SA) Boer, Kalahari Red and Savanna breeds and three dairy breeds consisting of the Saanen, Toggenburg and British Alpine. According to historical evidence, the meat breeds originate from indigenous goat breeds believed to have migrated to Southern Africa around 500 AD [3]. The Khoisan, a local tribe, moved with their herds southwards from Northern Botswana down to the Orange River from where two additional routes were used to reach the Southern and Western Cape. These goats kept by the local people were described by the Missionary J. Burrow as "handsome goats, speckled like the leopard" [4]. The indigenous goats most likely provided the genetic basis for the development of the current meat goat breeds. In contrast with the goat meat breeds, the dairy breeds have been imported from Europe and the United Kingdom. Besides these recognized goat breeds, South Africa has a large variety of indigenous or unimproved types that contribute meat, hides and milk to smallholders and subsistence farmers [5]. The majority of commercial goat farming takes place in the eastern and northern regions of South Africa where the species is well adapted to the vegetation [2].

2.1. Angora goats

The Angora goat was domesticated in Turkey, from where the animals were exported to Europe during the sixteenth century in an attempt to establish a rival mohair industry. The European climate was however not suited to these goats, and South Africa (a British colony at the time) presented a suitable region for Angora goat production. The first Angora goats were imported to South Africa during 1838, followed by another 3000 goats between 1856 and 1896 [6]. The Karoo and semiarid Eastern Cape region proved to be well suited to the Angora goats, and currently, the mohair industry in South Africa consists of approximately 644,000 Angora goats (www.mohair.co.za), most of which are still farmed with in the Eastern Cape.

The Angora Goat Breeders' Society was established in 1892 and is known today as the Angora Ram Breeders. The Angora goat is a relatively small, horned mohair goat with heavy and drooping ears, as shown in **Figure 1**. The hair and body of these goats are white, and an excess of color in the horns, hooves, ears and the skin is not allowed. An Angora goat should have a uniform fleece with regard to length and fineness, with good luster, solid style and good character, and should also be free from kemp or colored fibers [7].

Figure 1. A typical South African Angora doe and kid (University of Pretoria).

2.2. Meat goats

The South African Boer goat has the oldest official history with the establishment of the South African Boer Goat Association in 1959 [4]. In the development of goat breeds during the late sixties and early seventies, the Boer goat breeders' society referred to five potential types of Boer goat in South Africa [8]. These included unimproved types such as the ordinary goat, long haired types and polled types that originated from crossbreeding with dairy breeds and native goats. The improved Boer goat was recognized as the desirable type, breed standards were formulated and a number of goat breeders commenced with directional selection and a well-defined breeding policy that resulted in the modern SA Boer goat found in commercial and other farming systems today.

This breed is characterized by the red color of the head, long ears and a white soft coat (**Figure 2**). A sturdy head with a compressed nose and strong horns that have a gradual backward curve are favored. The goats have fleshy, well-developed broad briskets, well-sprung ribs, broad backs and muscular legs [9]. Mature Boer goat bucks weigh between 110 kg and 135 kg, and does weigh between 90 kg and 100 kg [10, 11]. The SA Boer goat does are known for their good mothering ability and can kid every 7–8 months. Some literature indicates a lower susceptibility to diseases such as blue tongue, prussic acid poisoning and, to a lesser extent, enterotoxaemia [8, 10].

The origin of the Kalahari Red and Savanna goat breeds is not as well documented and according to available literature probability originated from indigenous goat types [4]. These breeds have only been officially recognized in South Africa in 1990 and 1993, respectively. The

Figure 2. Typical South African Boer goats with white body and red head and neck (University of Pretoria).

Figure 3. A Kalahari Red goat with the characteristic uniform red coat color (University of Pretoria).

Kalahari Red has a dark red coat color and fully pigmented body that provides the advantage of high UV radiation tolerance. The Savanna goat is white in color and has short kempy hair with a black skin, horns, nose and udder [11]. These goats are also known to have well-muscled forequarters with a long neck for easy browsing. Typical Kalahari Red and Savanna goats are shown in **Figures 3** and **4**, respectively.

In **Table 1**, a summary is provided of descriptive measurements analyzed for the three commercial meat goat breeds. These breeds have been fully commercialized with official structures such as individual breed societies and well-defined breed standards. They are the main contributors to the official goat meat produced in South Africa [12] and are recognized for their superior growth and carcass traits [1].

2.3. Indigenous veld goats and Tankwa goats

South African indigenous goats are mostly characterized based on color variations and phenotypic characteristics such as ear length and horn shape. There is virtually no distinct breed identification, and populations are often named or identified according to the geographical region where they are kept. Various types are known, such as the Pedi, Nguni and Xhosa Lop ear ecotypes [1]. These uncharacterized veld-type goats generally have small body frames (mature females weigh approximately 40–50 kg) and low carcass yields. The goats are multipurpose and are used for meat, hides and sometimes even milk for younger children, mostly in small farming systems and or for household food production.

The indigenous veld-type goats have been subjected to limited selection and are largely unimproved genotypes. They have however contributed to the development of the local meat-type goats such as the Boer goat [4] through crossbreeding. The indigenous types vary in size and

Figure 4. A herd of Savanna goats with primarily white coat color (University of Pretoria).

	Body measurements in cm (least square means ± SE)		
	Boer goat	**Kalahari Red**	**Savanna**
Height (H)	56.5a ± 0.5	54.1a ± 0.5	55.7a ± 0.5
Length (L)	68.2ab ± 0.8	69.8a ± 0.8	64.9b ± 0.7
Depth (D)	26.4ab ± 0.3	27.1a ± 0.3	24.9b ± 0.3
Heart girth (HG)	90.3ab ± 1	95.3a ± 1	86.5b ± 1
Hock length (HL)	28.3a ± 0.5	27.7a ± 0.5	23.5b ± 0.5
Head width (HW)	7.7a ± 0.2	6.6b ± 0.3	5.6c ± 0.2
Head length (HL)	17.2a ± 0.3	15.7b ± 0.2	15.8ab ± 0.2
Neck circumference (N)	48.3a ± 4	42.5ab ± 3.8	37.7ab ± 3.7
Tail length (TL)	12.2ab ± 0.3	13.2a ± 0.3	13.3a ± 03
Pelvic width (PW)	13.8a ± 0.3	11.1b ± 0.3	11.4b ± 0.3
Pelvic length (PL)	19.7a ± 0.4	20.5a ± 0.3	19.1a ± 0.3
Ear length (EL)	21.4a ± 0.3	19.2b ± 0.2	19.5b ± 0.2

Table 1. Description of body measurements (cm) recorded for Boer goats, Kalahari Red and Savanna goats (adapted from Ref. [11]).

are often promoted as having special adaptive characteristics, including a higher tolerance for tick-borne diseases compared to the commercial goat breeds [8, 10]. In **Figure 5**, a typical South African veld goat is shown.

Figure 5. A herd of South African unimproved veld goats (Rauri Alcock).

The Tankwa goat refers to a population of feral goats that are found in the Northern Cape. Although they have been known to roam the Tankwa National Park for at least 80 years, they have only been identified and studied as a distinct population over the past decade. Their current population size is estimated at approximately 200 goats [1]. These goats have survived and reproduced in one of the harshest climatic regions of South Africa with regard to temperature and vegetation and hold potential for their unique adaptive traits.

2.4. Dairy goats

Dairy goats were introduced to South Africa at the turn of the twentieth century, originating primarily from Switzerland and the United Kingdom. The SA Mich Goat Breeders' Society was formed in 1958 but formal milk recording for dairy goats only started in the 1981/82 production year as the number of lactation records was low and variable before then [13]. Originally four breeds were officially recognized in South Africa, namely the Saanen, Toggenburg, British Alpine and an Anglo-Nubian Swiss composite [29]. Currently, the milk goat breeds include Saanen, Toggenburg and British Alpine breeds and crosses between these breeds are often used in commercial milk goat production systems [29]. The Saanen breed was the first milk goat breed to be imported to South Africa in 1898 [13] and is known for its high milk yield. The Toggenburg and British Alpine were imported during the early 1900s. These two breeds with dark pigmentation are favored for their adaptability to the climatic challenges of South Africa that includes high average temperatures and UV intensity. The Toggenburg furthermore also produces milk with a higher butterfat compared to the Saanen [13], which is important for production of cheese.

3. Quantitative selection and genetic improvement

Genetic improvement of small stock in South Africa can largely be attributed to the research performed over many decades in official research and prestige flocks. The results from the research flocks set the trends for selection and breeding programs, while the prestige flocks confirmed the value of applying a scientific approach to the farming community [14]. Performance recording was introduced for small stock, including goats, as early as 1956 by the Department of Agricultural and Technical services. Since then, production systems and environments have evolved and new selection tools became available for additional measurements, e.g., for fiber traits [15]. Advancements in the statistical methodologies for genetic evaluations made estimated breeding value possible for the breeds where sufficient animal and pedigree recording has been performed [14].

The genetic improvement of goats has been slow and less spectacular compared to sheep and other livestock species in South Africa. Of the goat breeds, most of the genetic improvement took place in the Angora goat due to the high economic value of mohair and South Africa being one of the largest producers of mohair in the world [16]. The poor participation in the National Small Stock Improvement Scheme (NSIS) by the meat and dairy goat breeders limits the potential genetic improvement, as limited phenotypic and pedigree recording occurs. Several factors play a role in the relative poor participation of SA goat breeders in animal

recording, including difficulties in recording on large extensive farming units, multi-sire practices presenting challenges for accurate parentage verifications and the cost of using modern technology for measuring traits of economic importance. Beef remains the primary choice for meat consumption by the consumer, and goats have often been neglected in the creation of new markets and products. All these factors may play a role in decision making by farmers when it comes to the costs involved in official animal recording and genetic evaluations. Furthermore, the unimproved veld goat is largely uncharacterized and has not been subjected to artificial selection or improvement strategies. It presents opportunities to utilize these goat types for improvement of the broader goat population due to their unique adaptive traits, but at the same time poses a danger if the selection strategies are not well formulated and implemented. Care must be taken that the uniqueness of genetic resources is conserved while implementing genetic progress.

3.1. Angora goats

The most significant genetic improvement in the South African Angora goat population took place over the past four decades. Although the National Small Stock Information Scheme was established in the 1950s, the uptake by Angora goat breeders was slow. A pilot study for animal recording in Angora goats was only implemented in 1983 [17]. The participation of Angora breeders in this scheme was voluntary and has remained poor over the past few decades. A lack of complete data for South African Angora breeders [18], combined with challenges regarding parentage verification, currently limits the application of breeding values.

In 1988, a research flock was established with the aim of breeding fine-hair producing Angora goats, without sacrificing body weight [14, 19]. Selection indices were made available to the breeders with emphasis on fiber diameter, fleece weight and body weight in varying ratios [20, 21]. This selection strategy resulted in a significant improvement of the fiber diameter and the general fitness of the Angora goat population [19].

The development of Optical Fiber Diameter Analyzer (OFDA) technology was important for obtaining accurate measurements for the full fiber profile. It has been implemented since 1992 in routine fleece measurement in South Africa by a number of breeders [22]. The quality traits associated with the full diameter profile (including coefficient of variation of fiber diameter, comfort factor and spinning fineness) hold potential for inclusion in the breeds' selection indices. In **Table 2**, a summary is provided of available heritability estimates for fiber quality traits in SA Angora goats [15].

Unfavorable genetic correlations between fiber diameter and fleece weight remain a challenge [22], and higher participation in recording and genetic evaluations will be required for further genetic improvement.

3.2. Meat goats

Most of the available research on meat goats was performed on the SA Boer goat, focusing on phenotypic characteristics [10] and production traits [8, 10]. Average reproductive performances for the Boer goat are reported [10] based on records obtained over a 20-year period, included a kidding rate (kids born/does mated) of 189%, fecundity of 210% and a weaning rate

Trait	h^2
Fleece weight (kg)	0.19 ± 0.04–0.24 ± 0.03*
Fiber diameter (μm)	0.26 ± 0.05–0.45 ± 0.03*
Coefficient of variation of fiber diameter (μm)	0.37 ± 0.10**
Standard deviation of fiber diameter	0.32 ± 0.11**
Comfort factor (%)	0.63 ± 0.11**
Spinning effective fineness	0.61 ± 0.10**
Standard deviation of fiber diameter along the length of the staple (μm)	0.14 ± 0.08**

* Snyman and Olivier [21, 24]; Visser et al. [22].
** Visser et al. [22].

Table 2. Heritability estimated for fiber quality traits in SA Angora goats obtained from OFDA measurements.

of 149% with a weaning weight of 29 kg at 120 days. The SA Boer goat has also been found to be early maturing with a high incidence of multiple births. Approximately 56.5% twins, 33.2% triplets and 2.4% quadruplets born were reported in a study on the influence of age on the reproductive performance of the improved Boer goat [8]. The high fecundity poses some obvious advantage under optimal feeding conditions, but could also result in increased kid mortality when reared under extensive conditions, especially with kids born as triplets and quadruplets. Some genetic progress is evident in growth traits as can be seen in the increase in 100-day weights based on performance of tested goats corrected for age and birth status from 1998 (25.3 kg for males to 22.3 kg for females) to 1996 (26.9 kg for males and 23.4 kg for females) [8].

Despite the availability of animal recording for small stock, the participation remains poor with only 38% registered Boer goat, 41% Kalahari Red and 67% Savanna goat breeders taking part in the Logix recording system for small stock [24]. Only one indigenous goat veld goat breeder takes part in recording out of 14 registered breeders. **Figure 6** highlights the poor participation of meat goat breeders in the NSIS.

The poor participation in animal recording of meat goats limits the potential for estimation of genetic parameters for traits of economic importance. In **Table 3**, available heritability estimates are presented for reproductive and growth traits. The available records for postweaning weights in South African Boer goat were insufficient for estimation of heritability [13]. A heritability value of 0.45 was reported for yearling weights in Australian Boer goats [25].

Selection progress for preweaning weights is likely to be slow due to low heritability estimates, whereas postweaning growth tends to exhibit higher heritability as seen in most farm animal species. The challenge for genetic improvement in the SA meat goat breeds lies in obtaining more and accurate recording for larger numbers of registered animals. This will enable genetic evaluations for breeding value estimation that can be applied by individual goat breeders in their herds as well as improvement of the national flock. A number of studies have highlighted the meat characteristics of South African Boer goat [28], but no genetic parameters are available for selection for improved carcass traits.

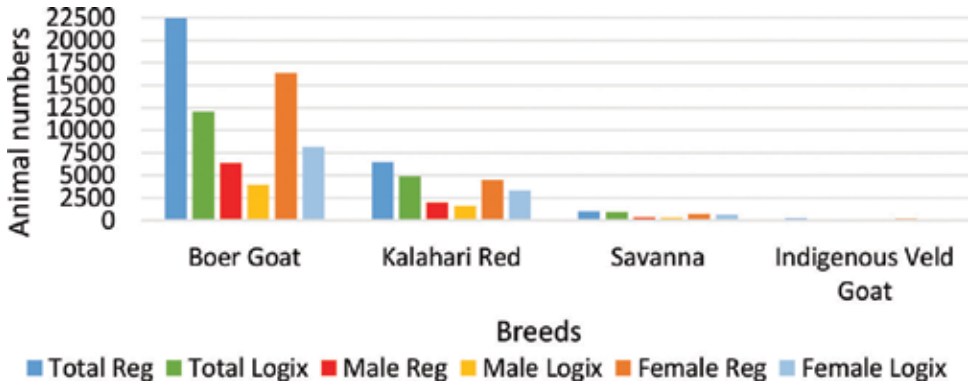

Figure 6. Participation of meat goat breeders in the National Small Stock Improvement Scheme [24].

Trait	h^2	References
Birth weight	0.05–0.14	Schoeman et al.[26]
Weaning weight direct	0.18–0.15	Van Niekerk et al. [26]; Schoeman et al. [27]
Weaning weight maternal	0.05–0.45	Van Niekerk et al. [26]; Schoeman et al. [27]
ADG	0.170	Schoeman et al. [26]

Table 3. Heritability estimates for preweaning weights of Boer goats.

3.3. Dairy goats

The South African dairy population is small in comparison with the other goat breeds and small stock. There are currently 45 registered herds representing 16,561 animals [24], and the remaining animals are used in commercial milk operations. Of the 45 herds, 16 herds (approximately 1217 goats) participate in official recording. Although participation in official animal recording is limited, the opportunity is available to record milk yield, milk composition and linear traits for selection and improvement. Heritability estimates have been reported for the SA Saanen for milk yield (0.23), butter fat yield (0.22) and protein yield (0.20). Protein and butterfat percentages had a heritability of 0.44 and 0.21, respectively [29].

Despite this relatively small population size, a niche market is served with the production of fresh milk and specialty cheeses. Marketing of these products occurs mostly in an informal way, such as by selling directly to consumers via on-farm sales, or at various markets. The renewed interest in organic products and dairy goats in general may result in breeders adopting modern technologies to overcome limitations in parentage recording and thus improved recording in order to perform accurate selection for long-term genetic improvement.

4. Molecular research and genetic improvement

Since the advent of molecular genetics, research on goats has entered a new era, also influencing the South African goat populations. The first research on South African goats was performed using microsatellite markers in the early 2000s and mainly involved genetic diversity and characterization studies. Genetic characterization assists in the conservation of unique characteristics of indigenous populations, whereas genetic diversity has a direct influence on genetic progress, selection strategies and the control of inbreeding levels. The identification of quantitative trait loci (QTL) explaining significant fractions of the genetic variance in economically important traits could lead to increased accuracy of estimated breeding values (EBVs) with a corresponding faster rate of genetic improvement. A few QTL identification studies were performed on Angora goats, but the limited amount of variation explained by these fragments restricted the application of the results in terms of marker-assisted selection (MAS). Some effort has gone into sequencing genes of economic importance and estimating their population frequencies as well as identifying novel variants in the local populations. The first caprine single nucleotide polymorphism (SNP) chip became commercially available in 2012, and since then (as with almost all other livestock species) SNP markers have become the marker of choice.

4.1. Angora goats

Without a doubt, the Angora goat breed is the South African goat breed on which most molecular research has been performed. The SA Angora goat served as the model breed for improving the goat linkage map in 2010, using 94 microsatellite markers [30]. Both the accuracy and the coverage of the map were improved by adding markers, correcting previously reported order alignments and decreasing map distances. This linkage map formed the basis for a number of studies performed on the SA Angora goat.

Angora goats in South Africa are primarily farmed extensively and are subjected to group mating and over-mating. This limits accurate parentage recording and has a negative effect on the accuracy of estimated breeding value estimation and selection progress. A DNA parentage verification panel was created, using 14 microsatellite markers with a combined probability of exclusion of 99.7% [31]. The impact of DNA-based parentage verification on EBV accuracies and ranking of sires were evaluated a few years later [32]. It was shown that correct allocation of parentage had a significant effect on EBV estimation and ranking of sires, especially for growth traits. DNA-based parentage verification enhanced selection accuracy and would result in faster genetic progress.

Phenotypic recording and EBV selection on mohair and growth traits were relatively successful during the 1980–1990s. However, intense selection pressure for increased mohair quality and yield resulted in small, unthrifty goats with high mortality rates. QTL identification studies were performed to identify chromosomal segments associated with product and quality traits of mohair [33] as well as preweaning growth [34]. Eighteen QTL for mohair traits (including fleece weight, fiber diameter, coefficient of variation of fiber diameter, comfort factor, spinning fineness and variation along the length of the fiber) were identified on 13 chromosomes [15]. In the study focusing on preweaning growth traits, four chromosomal regions of interest with an influence on birth weight were identified on CHI 4, 8, 18 and 27

and two candidate regions for weaning weight on CHI 16 and 19, respectively [34]. Although putative QTL were identified in both studies, the QTL explained limited phenotypic variation of the traits, which is one of the main restrictions of marker-assisted selection. No MAS has yet been implemented in the SA Angora goat breed.

The QTL identification study did however indicate that QTL associated with mohair production and quality were located on chromosomes where the KRT and KAP genes have previously been assigned to mainly CHI 1 and 5 [33]. Polymerase chain reaction (PCR) and sequencing technology were used to identify and characterize KAP 1.1, KAP 8.1 and KAP 13.3 in South African Angora, Boer and Angora x Boer goat populations. A total of 19 novel variants were identified in total, and in these, three genes were responsible for structure and quality of hair fibers. The predominant alleles differed between the various populations and together with high levels of observed heterozygosity hold promise for selection based on favorable allelic associations [35].

The development of a moderate-density genotyping tool, the 50K SNP chip (Illumina Inc., San Diego, CA) [36], was a key milestone for molecular research in goats. Due to the fact that no fiber-producing breeds were included in the development of this commercial chip, it was first validated in the SA Angora goat population [37]. Fortunately, the high level of polymorphism observed (88.1% of loci) and the sufficient observed heterozygosity levels in the population (0.365) made the bead chip suitable for application in this breed.

The 50K SNP chip was subsequently used to estimate genetic diversity in the SA Angora goat. Results indicated that sufficient genetic diversity still exists within this breed to allow successful selection strategies and genetic improvement [38]. A high proportion of SNP with low minor allele frequency (MAF) values suggested a high proportion of fixed alleles, which was in line with the high selection pressure on specific traits within this population. An linkage disequilibrium (LD) estimate (using the r^2 measure) of 0.15 was calculated, which implied that a denser SNP genotyping array would be necessary before genomic selection (GS) could be considered for the SA Angora.

The SA Angora goat was included in a study to analyze the genetic variability of Angora goats from three distinct geographical locations (South Africa, France and Argentina) in order to assess the influence of genetic and geographical isolation [39]. The fixation index (FST) indicated three distinct subpopulations, with intrapopulation values (0.12) corresponding to those normally observed between breeds. An effective population size (Ne) of 93 was estimated for the SA Angora goat, 100 generations ago, and is currently probably even lower. The distinctiveness of the South African population indicated strict directional selection which has resulted in a well-defined cluster. The high diversity between populations could be useful when exchanging genetic material to improve certain unfavorable characteristics of specific populations.

4.2. Commercial and indigenous meat goats

Both commercial and indigenous goats have been included in studies where DNA markers have been applied to gain insight into their genetic diversity and population structure. However, significantly, less research in terms of molecular studies has been performed on meat goats than on the Angora goat breed.

The first molecular study on SA meat goats was performed in 2004 when the genetic variation of the three commercial breeds as well as three indigenous goat populations were investigated using microsatellite markers [40]. A clear differentiation between the Kalahari Red and Boer goat breeds was observed, whereas the Savanna breed showed significant genetic similarity to the Boer goat. Limited differentiation was observed between the veld goat populations, as was expected. Of all the breeds and populations, the Kalahari Red breed was the most clearly differentiated on a genetic level. The distinctiveness of the Kalahari Red breed was further investigated [41], also using microsatellite markers. Although it appeared that the breed was largely uniform, limited differences suggested local selection and adaptation. The clear genetic differentiation of the Kalahari Red breed was confirmed by a later study focusing on only commercial goat breeds [11]. A factorial correspondence analysis was performed with microsatellite data and the Kalahari Red goats clustered on their own, while the Boer goat and Savanna populations tended to overlap.

The commercial Boer goat and Kalahari Red breeds, as well the Tankwa and two indigenous populations, were included in a study to characterize African goat populations using the Illumina Goat SNP50K genotyping array [42]. These South African breeds showed a higher level of variation when compared to other African populations. Preliminary results were reported by [43, 44] on the population structure and landscape genomics of indigenous goats using genome-wide SNP data. The goat populations showed sufficient genetic diversity, and the Tankwa population was revealed as a distinct breed. Associations between the genomic variation of the goats and climatic conditions were limited to associations with longitude, temperature and altitude using the spatial analyses method.

The genetic architecture of the three commercial meat breeds, the Tankwa and five distinct ecotypes (Nguni, Venda, Xhosa, Zulu and Tswana) were investigated by Ref. [45]. Ecotypes were found to have the highest levels of genetic diversity and low levels of inbreeding, probably due to the lack of directional selection in communal systems. Most of the ecotypes showed some level of genetic relatedness with one another. The Tankwa breed was again identified as a unique genetic resource with low genetic diversity and high inbreeding levels, which can be attributed to the small population size and geographical isolation of these animals.

4.3. Dairy goats

The Saanen, British Alpine and Toggenburg are the three breeds contributing to South Africa's small dairy goat industry. Most goat milk is processed and sold as goat's cheese; thus, the quality of the milk produced and specifically the casein content is of importance. Limited molecular research has been performed on these breeds.

To date, two studies have been performed to characterize casein in the SA goat breeds, one on κ-casein [46] and another on αS2-casein [47]. The first study investigated indigenous, Boer and Saanen goats using restriction fragment length polymorphism (RFLP) and DNA sequencing. Two less favorable alleles (B′ and H) were found exclusively in the meat goat populations, while the favorable B allele was fixated in the Saanen goats. In the latter study, αS2-casein was genotyped in the three SA dairy breeds, as well as in some meat-type goats using DNA sequencing. Four alleles and 10 genotypes were observed across the

populations, with the A allele being the most frequent in all the breeds. Limited gene-specific selection opportunities are possible based on these results.

The genetic diversity of SA dairy goats was investigated using a panel of 25 microsatellite markers [48]. High levels of diversity were estimated in all three breeds, with heterozygosity values exceeding 60%. Limited inbreeding was observed within the populations. The genetic differentiation between the dairy breeds was very low, as could be expected within one production type. An admixture group of animals was identified, suggesting that inadvertent crossbreeding between purebred animals was taking place. The SA Milch Goat Breeders' Society allows the registration of goats with unknown pedigree, based on a physical inspection (mainly color pattern and functional efficiency). It has however been clearly demonstrated that coat color is not a definitive way of assigning breed status. Some dairy goats were included in the SNP-based genetic diversity study by Ref. [38]. The results corresponded with that of the previous study, with relatively high gene diversity estimates within the breeds. A 30% co-ancestry was calculated between the breeds, supporting the previous findings [48] regarding admixture.

5. Conclusion

The various goat breeds and populations in South Africa serve a number of purposes ranging from important economic contributions to the commercial livestock production sector, to the improvement of livelihoods and food security in rural communities. Genetic progress can primarily be attributed selection following a quantitative approach, with a focus on fertility, growth and some breed-specific production traits such as fiber yield. Future research and selection for genetic improvement will most likely be targeted toward molecular-based approaches. Molecular research has shown that most SA goat breeds have sufficient genetic diversity to be exploited in selection programs. Specific projects are targeted toward the identification of genes associated with traits of economic importance, managing inbreeding levels and sustainable conservation and utilization of scarce genotypes.

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Nutrition

Chapter 3

Rate of Passage of Digesta in Ruminants; Are Goats Different?

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Additional information is available at the end of the chapter

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Abstract

Fluid passage rates through the rumen influence digestion of soluble food nutrients, amount of short-chain fatty acids absorbed in the rumen and that pass out of the rumen, the amount of by-pass protein of dietary origin and the amount of microbial protein available to the host as a protein source, making modelling of passage imperative. Current research on passage rate should seek to incorporate various factors that affect rumen fill, and solid and liquid passage rates to develop intake and passage rate prediction models. The aim of this paper was to discuss factors that affect rates of passage of digesta and rumen digesta load. Ambient temperature, animal physiological status and reproductive status, fermentation and diet quality are major factors affecting digesta passage rates. The animal physiology also influences digesta passage rate. Computation of animal production level to account for all the physiological processes that affect passage rate is vital. Discrepancies on how ambient temperature and particle density (buoyancy) affect the passage rate of digesta in the rumen may cause uncertainty in calibration of temperature and buoyancy in prediction models. Corrected for diet properties, goats have similar passage rates to other ruminants.

Keywords: diet selection, feeding behaviour, intermediate feeder, prediction model, ruminant

1. Introduction

Goats have become one of the most important livestock for resource-limited farmers around the world because they can survive in harsh climatic conditions from cold temperate regions (in the Siberia) to hot arid deserts (in the Kalahari). Key to their ability to survive in diverse climatic regions is their ability to walk through tight, narrow places and their capability of efficiently utilising vast plant feed resources. Goats are important as a source of protein (milk and meat) and wealth. Given the increasing importance of goats among resource-limited farmers in tropical

and subtropical regions around the world, research on the physiological aspects of goat nutrition is vital for improved goat production. Feed availability and quality are the major factors affecting yields for and quality of chevon and milk in rural goat production systems. Enhanced milk and chevon productivity is largely dependent on their selective feeding behaviour supported by improved nutritional status through supplementation of poor-quality roughages with feeds of high nutritional value (e.g., protein and energy concentrates). Concentrates are generally expensive for resource-limited farmers in rural communities around the world, especially in Africa and Asia. Hence, cost-effective usage of these feeds is vital. Fundamental to cost-effective concentrate supplementation for improved productivity of goat farming systems in the tropics relies on accurate and precise prediction of roughage intake. Accurate prediction of roughage intake would enable farmers to calculate precise quantities of concentrates to be fed to achieve a cost-effective level of production of good-quality meat and milk.

One of the major challenges in developing sustainable and cost-effective feeding strategies for goats in rural production systems in Africa, Asia, and other parts of the world is the inability to accurately predict roughage intake in goats. This is partly due to limited information on the critical factors that affect intake, passage rates of digesta and rumen fill for goats. Nsahlai and Apaloo [1] examined the appropriateness of model in [2] to predict the intake in ruminants grazing on poor quality roughages in tropical regions and showed that the model, though structurally adequate, underestimated roughage intake, partly due to poor estimates of gut fill and rate of passage. Similarly, the authors [3–5] showed that the model of Illius and Gordon [2] overestimated retention time in browsing ruminants for particle sizes less than 2 mm. Nsahlai and Apaloo [1] pointed out that the model of Illius and Gordon [2] erroneously estimates rumen fill levels and passage rates as a function of body weight alone.

Given the role of fluid passage rates through the rumen in affecting by-pass proteins and fatty acids that are assimilated in the hindgut, passage of fluid would have a significant influence on milk protein and butterfat composition in dairy goats. This makes the study and modelling of digesta passage rate relatively important. Modelling of passage rates would necessitate prediction of roughage intake, microbial protein yield and milk composition in goats. Before any passage rate modelling exercise can be effectively carried out, factors that affect passage rates need to be reviewed and used to predict digesta passage rates.

Given the abundant literature on passage rates for cattle and sheep, and other ruminant feeding types, and limited data on goats, the chapter (i) identifies the major factors that affect passage rates in ruminants, (ii) explains the fundamental mechanisms by which each identified factor possibly affected rates of digesta passage from the rumen, (iii) gives suggestions of the major factors that can be considered as critical input factors for developing passage rate prediction models distinguishing studies on goats and (iv) determines whether goats are different with respect to other ruminants in terms of passage rates.

2. Rumen fill, gut capacity and its estimation

Gut fill is referred to as rumen fill with respect to ruminants based on the facts that the rumen is the only site in the gastrointestinal tract where distension has an effect of restricting digesta

flow to a great extent [6]. Maximal rumen load for dry matter is determined by allometric procedures as a function of body weight [1, 2]. However, a ruminant's fill capacity also depends on the volume of digesta that causes rumen distension and on rate of flow of digesta from and rates of degradation of digesta in the rumen [6, 7]. As a result, criticism on determination of rumen fill based on body weight alone has been raised giving better models for rumen load based on body weight, mature body weight and dietary crude protein [1]. This fill capacity may also be determined practically by manually emptying the rumen at a time when full gut capacity is reached and weighing out digesta at that time [8] or slaughtering animals upon meal termination [9]. Rumen fill varies greatly with body weight and feeding habit. Rumen fill is approximated to be about 9 and 13% of body weight for browsers and grazers, respectively [8]. Interestingly, there is no apparent approximation of rumen fill based on body weight for intermediate feeders such as goats, although it may be assumed to fall within the range of 9–13% when grazers and browsers are regarded as extremes.

Carrying out rumen evacuations to determine maximal rumen digesta load is not an easy task. It is assumed that the only or best way to know when an animal has reached its maximal gut capacity is when it stops or terminates feeding [10]. This theory is supported by Boudon et al. [11], where termination of short-term feed intake was attributed to signalling from the rumen wall as a result of rumen fill. Taweel et al. [12] and Williams et al. [13] reported scenarios whereby maximal rumen fill was greater after termination of late afternoon feeding bouts just around sunset than bouts from other parts of the day. According to these results, measuring maximal rumen fill after termination of morning feeding maybe misleading. However, Baumont et al. [14] reported rumen fill to reach its first maximum after the main morning meals, with a daily maximal rumen fill being reached after the evening meal. Similar results were obtained by Thomson et al. [15], where maximal rumen fill was observed after termination of first morning meal and late evening meal at 0900 h and 2000 h, respectively, in grazing sheep. Assuming that at meal termination, ruminants would have reached maximal rumen capacity is misleading as well. This assumption is supported by Taweel et al. [12] and Chilibroste et al. [16] who reported findings where maximal rumen capacity had not been reached when grazing dairy cows terminated feeding bouts. Greenhalgh and Reid [17] reported similar results where sheep fed on hay and straw-terminated feed intake way before maximal gut capacity was reached. These and other experimental results therefore suggest the existence of different sets of rumen fill levels which are time dependent, either before or after feeding bouts. These fill levels may be a function of the rate of emptying of rumen digesta after eating has stopped. At any given time, rumen fill levels are a function of the rate of feed intake, rates of digestion and particle breakdown, and rates of outflow [13]. As such, rumen fill levels or values are dynamic and thus should not be regarded as constants and times in which rumen capacity is measured should be taken into account as well.

Based on rates of passage and digestion, estimation of rumen capacity using mathematical procedures gives variable but useable results. Estimated rumen pool size based on passage and degradation rates at the point of meal termination gave values which were even twice as large when compared to average observed values obtained from the literature (Nsahlai, unpublished data). Failure of mathematical procedures to achieve tenable outcomes suggests that something uncertain takes place during the period after meal termination before evacuation.

3. Factors that affect rumen fill levels and rates of passage through the rumen

3.1. Animal species and feeding types

Ruminant livestock has different feeding habits [4] with cattle, buffalo and sheep classified as grazers and goats as browsers or intermediate feeders [18]. Differences in type of diets and processes associated with feeding behaviour between these classes of animals may have an effect on rates of passage of liquid and solid phases in the rumen [19] and their rumen fill.

Sheep had lower mean retention times of solid in the rumen than cattle (58 vs. 65 h) [20]. Lechner-Doll et al. [21] added that selective retention of particles is more pronounced in cattle than in sheep suggesting that the passage rate of large particles is greater in sheep than in cattle. Outflow rates of fine solid material are normally estimated by allometric procedures as an inverse function of body weight, which infers erroneously that the rate of passage in smaller ruminants is always greater than that in larger ruminants [1]. Parra [22] showed higher passage rates for smaller herbivores than larger herbivores with diet quality held constant. Differences between cattle and sheep with respect to solid retention times depend on chewing activities of these species. Average chewing rates are higher in sheep (80–100 chews per minute) than in cattle (40–60 chews per minute), indicating different efficiencies in these ruminants. Consequently, cattle have developed pronounced selective retention mechanisms for large particles in the floating fibre mat found in the dorsal rumen to improve particle size reduction and perhaps nutrient extraction; hence, retain particles for longer periods compared to sheep [23, 24].

Oshita et al. [25] reported differences in passage rates and rumen fill levels among cattle as a result of different grazing strategies. Rumen fluid dilution rates were higher for rotational grazed cattle (12.2%/h) compared to cattle fed pasture in confinement (9.9%/h). Similarly, rumen volume was lower for rotationally grazed cattle (79.9 l) compared to cattle fed in confinement (110 l). Williams et al. [13] showed no variations in rumen pool sizes with pasture allowance and time of day. Lack of differences in rumen fill levels with increasing pasture allowance is due to great variations in outflow rates.

Great variation in passage rates between animals of the same class of ruminants may occur as a result of differences in feeding habits. Although very little or no evidence for this phenomena has been documented, it is highly likely to occur. Dorper sheep are less selective of feed, consumed more shrubs and bushes than Merino sheep during grazing in the Noorsveld Karoo, South Africa [26]. Dorpers would be expected to have slightly faster passage rates than Merinos because they consume more browse. Hence, it would be expected that Dorpers spend less-time re-chewing twigs than Merinos resulting in more-intense rumen contractions that forced digesta out of the rumen quickly. Goats have a much similar feeding habit to these Dorper sheep as they utilise both graze and browse and are more selective of high-quality browse. It can be concluded that passage rate in ruminants is affected by interactions between diet, ruminant species and their climatic environment.

Molina-Alcaide et al. [27] observed no differences in particle passage rates in goats (intermediate feeder) and sheep (grazer) fed on various diets with average rates of 0.030 and 0.025/h, respectively. However, Clauss et al. [28] suggested that smaller browsing species had much greater solid and fluid passage rates through the rumen than grazers of a much similar size. A much different trend exists in larger individuals of each feeding habit. Larger grazers tend to show higher solid and fluid passage rates through the rumen than browsers of similar size [28, 29]. Surprisingly, intermediate feeders (such as goats) were not included in this comparison. Processes that occur in the rumen when different diets are fed coupled with the anatomy of the fore stomach associated with each ruminant feeding type are implicated in these differences. Fluid and solid passage out of the rumen occur through an opening between the reticulorumen and the omasum called the reticulo-omasal orifice [30]. Positioning and size of the reticulo-omasal orifice may shed insight on the flow of liquid and solid digesta from the rumen. Hofmann [18] showed that the size of the reticulo-omasal orifice was greater in browsers than in grazers. It may be hypothesised that due to the larger reticulo-omasal orifice in browsers, a much greater volume of solid and fluid passes through the rumen per unit time than in grazers. This may cause browsers to have higher fluid passage rates than grazers in smaller animals [30].

With respect to the larger groups of animals, grazers possess larger omasum than browsers [18]. One of the functions of the omasum is to absorb water [31], thus it may be logical to assume that there is a much greater water pulling effect (cohesion and capillary movement) of the grazers larger omasum than that of browsers. This could result in higher passage rates of fluids out of the rumen of grazers. Due to a greater receptive space of the omasum, the pressure difference between the rumen and omasum [30] is larger in grazers than in browsers. Hence, greater rates of passage of fluid observed in grazers may be due to a larger pressure difference. This may not apply to small grazing and browsing animals. Hence, a gap in knowledge on the relative sizes of the omasum in smaller grazers, intermediate feeders and browsers exists.

Indirect evidence suggests that browsing ruminants have shorter mean retention times for liquid and solid digesta in the rumen compare to grazers. These include postruminal absence of glucose transport mechanisms (GLUT transporters) in grazers which are present in browsers [4, 32, 33], deposition of large quantities of polyunsaturated fatty acids in browser carcasses compared to grazers [4, 34], lower efficiency of fermentation [4, 35] and total tract digestibility [36] in browsers than grazers and presence of large amounts of particles that are greater than 1 mm in faecal samples from browsers compared to grazers [3, 18, 37, 38]. These differences are partly a result of faster fractional passage rates of fluid and solid through the rumen of browsers compared to grazers.

Differences in viscosity of rumen fluid and saliva between grazers and browsers exist [39]. Browsers have more viscous rumen fluid [29] and saliva [18] than grazers. The thicker and stickier the fluid digesta may have an effect of reduced movement of the fluid through the rumen due to increased attachment of water molecules to feed particles. Hence, fluid is less likely to escape from the rumen thus resulting in reduced fractional passage rate

of fluid in the rumen of browsing animals. However, Silanikove et al. [40] obtained conflicting results to [29], where polyphenolic compounds increased the rate of fluid passage through the rumen. Polyphenolic compounds cause fluid digesta to be thick and sticky as a result of more viscous saliva production, which is a case in browsers [39]. Hence, viscosity of rumen fluid increases due to the presence of polyphenolic compounds. The expected outcome is decreased fluid outflow rate. Contrary to that, increased viscosity due to polyphenolic compounds may increase the rate of passage of fluid. Fluid from the interstitial spaces may be drawn into the rumen in an attempt to wash off these polyphenols [40] as a physiological response by the animal against them. This occurrence may then result to increased rates of fluid passage through the rumen.

Due to observed differences in passage rates among ruminant species, possible differences in rumen fill may be expected given that the passage rate is related to the amount of digesta in the rumen at any given time. Molina-Alcaide et al. [27] showed that rumen fill and amount of rumen contents are larger for goats compared to sheep. It was concluded that goats possessed a unique characteristic of being able to maintain larger rumen fill levels without noticeable rumen distension than sheep when fed medium-quality diets. These results were not expected taking into account observations by Clauss et al. [28] showing that smaller browsing species had much greater fluid and solid passage rates through the rumen than grazers of a much similar size, suggesting that goats should have lower rumen fills than sheep. Cattle are expected to have a much larger gut capacity than sheep and goats when scaled to body weight. Parra [22] showed that metabolic rate increased as a fractional power of mass (W^x) suggesting that small ruminants have smaller rumen capacity per unit metabolic need. Hence, as a result, cattle would be expected to have a greater rumen capacity than sheep and goats. Due to the abovementioned theories, small bodied ruminants with smaller gut capacity must compensate for this constraint by increasing passage rate to ensure they maintain adequate feed intakes to meet metabolic needs [41]. This may help explain why sheep had higher passage rates compared to cattle [20]. Body weight cannot be convincingly classified as a factor that affects mean retention time [42]. At body masses less than 100 kg, Wenninger and Shipley [43] showed in cattle that there was no relationship between the body weight and mean retention time.

Differences in passage rates among ruminants exist as a result of differences in habitats in which they live and are adapted, which is dependent on the type of diet available. Silanikove et al. [44] showed that average fractional flow rates tended to be lower for desert goats (0.084/h) than non-desert goats (0.099/h). This translated to +39% higher fluid passage rate in non-desert goats per unit body weight. Again, mean retention time of solid particles was 10 h greater for desert goats with intake being predominantly limited by high levels of rumen fill [44]. These findings indicated that desert ('tropical' or hot climate) goats may possess greater digestive capacity than other breeds of goats as a result of adaptation to feed and climatic conditions in the desert. Passage rate and rumen fill data for goats adapted to subtropical and tropical climates in sub-Saharan African are limited, thus necessitating data on how climatic adaptation influences passage rates and rumen fill.

Rumen capacity and fill levels at any given time vary according to breeds as well. Breeds better adapted to low-quality forages tend to possess increased rumen capacity for both

digesta phases. Weyreter and Engelhardt [45] found that Heidschnucken sheep (well adapted to high fibre roughages) were better able to consume large amounts of fibrous diets compared to Merino sheep (less adapted to high fibre roughages). This suggested that Heidschnucken sheep have greater potentials in expanding their rumen capacity compared to Merino sheep. Black head sheep (cold climate or temperate breed) are unable to make such an adaptation relative to Heidschnucken sheep [45].

A new theory on passage rate is beginning to unfold based on anatomical features of the rumen in different ruminant feeding type. Clauss et al. [46] suggested that digesta passage patterns are correlated to and influenced by intraruminal papillation patterns. Differentiation between grazers and browsers using papillation patterns characterised grazers as having long, thick papillae and deep reticular crests and ridges. Browsers characteristically have short and much thinner papillae and shallower reticular crests compared to grazers. Presence of deep reticuloruminal papillae and crests caused entrapment of small particles in ridges of grazing ruminants than in browsers, causing longer retention times in grazers [42].

3.2. Level of nutrition and feed intake

Plane of nutrition may be referred to as the level of feeding and animal production level. Level of feeding is defined as the amount of feed the animal consumes relative to its level of feeding to meet maintenance requirements [8]. Cases of hyperphagia increase demands for expanded rumen capacity so as to accommodate much greater digesta load [47]. Quantities of feed ingested by ruminants depend on animal species, and the variability in intake levels occurs between breeds and/or individual within a breed [48].

Haaland and Tyrrell [49] observed that the rates of passage of fluid through the rumen increased by 13% when animals were fed at two times maintenance from feeding at maintenance level. The authors [50–53] observed that an increase in dry matter intake was associated with linear increases in fluid passage rate. As an animal eats more dry matter, solid material entering the rumen accumulates, and there is a possibility of dry matter taking up space occupied by the fluid in the rumen thus exerting pressure on the rumen contents. With dry matter being more bulky [54] than liquid, there is a possibility of the bulk forcing liquid out of the rumen at a much faster rate as the pressure builds up in the rumen compared to low intake levels. In muskoxen, Barboza et al. [47] showed that elevation of feed intake by 74% increased gut fill by 31–34%. Hyperphagia increases gut fill, and gut fill is usually a result of reduced passage rate of solid material. On the other hand, this observation is inconsistent with studies where increased feed intake has been shown to increase passage rates. Although Lindberg [51] showed a strong relationship between liquid passage rate and feed intake in dairy goats, no correlation was reported between dry matter intake and mean retention time in addax [54]. This suggests that high dry matter intakes may not necessarily influence passage rates through the rumen. Long mean retention times for particulate matter at high dry matter intakes in addax may have been due to a high reserve capacity of the reticulorumen. Accurate determination of the extent to which rumen capacity may expand to accommodate various types of forage diets in different ruminants would be important. This elicits determination of maximal rumen fill levels in ruminants. Body weight had high positive

correlation to rumen capacity [55]. Distension of abdominal cavities during the projected increases in rumen capacity have not yet been quantified and documented in any species [56]. Estimates to which ruminant gastrointestinal tracts stretch to accommodate a given diet range roughly lies between 10 and 17% of the body mass in ruminants, with an upper limit of 20% for cattle. Goats and sheep reach this upper limit more frequently and easily than cattle [57]. Body weight alone is not a good indicator of maximal rumen fill; Purser and Moir [58] reporting variation in gut capacity among animals of similar body weight. Tulloh and Hughes [59] reported larger rumen volumes in lactating cows than dry cows. Hence, rumen fill or volume is more a function of various physiological states.

3.3. Forage-to-concentrate ratio in the diet

Supplementation of predominantly roughage-based diets has become a major practice in ruminant nutrition. Protein concentrate supplementation of ruminants grazed on pasture increases the nutritional status of ruminants [60]. Levels of concentrates added to predominantly roughage feed would affect the rate of passage of liquid and solid through the rumen. High roughage to concentrate ratio in the diet would lead to greater fluid and particulate passage rates from the rumen (**Table 1**). Passage rate is affected by roughage quality and the rate at which rumen digesta disappeared from the rumen is positively related to diet quality [1].

Bartocci et al. [20] reported an increase in passage rates of fluid and particulate matter from the rumen with an increase in the proportion of dietary fibre in diets fed to buffalo, cattle and sheep. All authors [61–65] reported that high proportions of concentrate in diets decreased the rates of fluid dilution and turnover in the rumen.

Although similar trends on the effects of forage-to-concentrate ratio on fluid dilution rate and fractional passage of solid were observed, a number of suggestions have been given toward explaining these observations. Forage-to-concentrate ratios can alter a number of processes in ruminants, and these processes have been implicated to changes in fluid and solid outflow rates from the rumen. These processes include the amount of saliva produced and the degree of stratification of rumen contents.

Froetschel [66] showed that cattle produced an average of 100–200 l of saliva in a single day when fed high-fibre diets. Saliva is mainly used as a buffering agent and lubricant as roughage digestion produces large amounts of short-chain fatty acids that may lower rumen pH.

Phase	**Diet**	**F:C = 87.5:12.5**	**F:C = 75:25**	**F:C = 62.5:37.5**	**F:C = 50:50**
	Parameter				
Liquid	OFR (l/h)	3.47	3.16	2.76	2.41
Liquid	RF (l)	49.10	46.10	43.60	40.00
Solid	k_1 (%/h)	3.15	2.71	2.71	2.48

F:C, forage-to-concentrate ratio; OFR, outflow rate; RF, rumen fill; k_1, fractional passage rate. Adapted from Ref. [20].

Table 1. Effect of forage-to-concentrate ratio in diet on rate of solid and fluid passage through the rumen.

Increased amount of saliva forces ruminal wall contractions to escalate [66]. An increase in these contractions may be stimulated by increased distension and tactile stimulation of the rumen wall due to filling by saliva. Distension of the rumen wall results to contractions, emptying the rumen fluid and solid digesta to prevent it filling. These contractions squeeze solid and fluid digesta out of the rumen at an increased rate increasing the efficiency of microbial protein synthesis. Bartocci et al. [20] observed decreased amounts of saliva production in animals fed high-concentrate diets that constituted 50% of the diet. Hence, reduced salivation may be responsible for lower passage rates in high-concentrate fed animals due to reduced rumen contractions. Another possibility is that occurrence of these increased contractions might be due to mineral ions present in saliva.

Due to the bulky nature of forage, high roughage diets may occupy a large space in the rumen. Bulky forage may force liquid out of the rumen at a much faster rate as competition for space increases. Tactile stimulation of the rumen wall by the roughage is a likely facilitator. Because of a much greater degree of tactile stimulation, rumen wall contractions may occur, thus forcing rumen fluid to pass through the rumen at a much faster rate. Okine and Mathison [67] showed that an increase in duration and amplitude of reticulorumen contractions resulted in an increase in passage rate of both solid and liquid matter out of the rumen. Due to less bulk, concentrates would occupy far much less space in the rumen than forages; thus, high-concentrate diets would induce low-amplitude rumen contractions due to reduced tactile stimulation of the rumen wall. Low power of contractions would force less fluid out of the rumen per unit time compared to roughages leading to lower fluid and solid passage rates. Rumen fill was reported to be greater in diets that had higher proportion of roughage because roughages contribute to rumen fill more than concentrates in view of longer retention times in the rumen and selective retention in the fibre mat. Lui et al. [68] observed reduced clearance rates of solid digesta in the rumen of animals fed bulky high fibre crop residues. Lui et al. [68] gave clear evidence of enhanced rumen fill levels as a result of high fibre/roughage content in ruminant diets. Concentrate particles are small and the chance of being trapped in the floating mat is minimal; thus, it passes out of the rumen at a much faster rate than roughage particles.

In the rumen, stratification occurs (**Figure 1**). Stratification involves separation of liquid and solid components into distinct layers according to density [69]. Stratification is evident when a mat-like layer forms and floats on the liquid phase. Fibre promotes the formation of the floating mat [70] in roughage more than concentrate diets because concentrate particles are smaller forming more homogenous mixtures in the rumen. Formation and presence of a floating mat in the rumen stimulates ruminal wall contraction [57], possibly due to tactile stimulation of the rumen wall. These contractions may lead to a rapid outflow of liquid

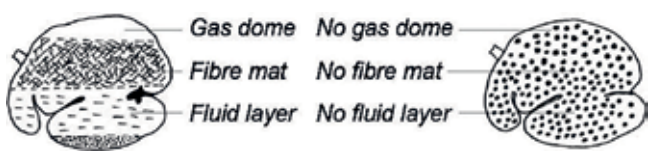

Figure 1. Proposed degree of stratification in rumen due to roughage (left) and concentrate (right). Adapted from Ref. [69].

and fine-solid digesta through the rumen. Faichney [71] showed that entrapment of large solid particles in the filter bed of the rumen restricted their outflow. Entrapment increases retention time of large particles; hence, fibre-mat formation may be a factor labelled as affecting rate of passage of solid through the rumen.

However, the theory of stratification may be challenged. Moore et al. [70] showed that cottonseed hull diets, even though fibrous and elicit a faster rate of liquid flow through the rumen, do not promote stratification. Hulls are smaller and denser and form a more homogenous-like mixture in the rumen [57]. Moore et al. [70] concluded that rates of fluid flow through the rumen increased because of increased intake of the hull diet. Contrary to that, Owens and Goetsch [72] reported that cottonseed hulls resulted in decreased passage rates of fluid in the rumen, thus supporting the theory of stratification. Further studies on the effect of cottonseed hulls on the rate of passage need to be done. The theory of stratification that supports increased flow rate of fluid through the rumen may be applied to higher passage rates in grazers than in browsers due to differences in diet. Grazers are mainly roughage eaters, and browsers are concentrate feeders [18]. Hence, higher rates of passage of fluid are seen in grazers than browsers.

Stage of development of forage may also have an effect on the fluid dilution rate and solid passage rate [55]. When a plant is young, it contains a higher proportion of water than old plants, with older plants tending to have a larger proportion of lignin. With older plants having large proportions of lignin than younger plants, it is expected that forages at a late stage of development may induce higher liquid passage rate. However, ruminants that graze on grass that is at an earlier stage of development have high fluid passage rates through the rumen than those grazing on mature pasture. Work by Adams et al. [55], Estell and Galyean [73] and McCollum and Galyean [74] showed that animals grazed on young pastures have higher dilution rates (18.3%/h) than animals grazed on mature pastures (9%/h). The presence of high mineral and water content in young forage may led to increased osmotic pressure in the rumen causing the relaxation of the reticulo-omasal orifice thus increasing fractional rates of passage of fluid through the rumen. Lignin and hemicellulose contents of forages may have a substantial effect on passage rates of both solid and liquid matter in the rumen. Mature forage contains a higher proportion of hemicellulose than young forages [75]. Hemicellulose has hydrophilic properties [76] and capabilities of absorbing and holding water in the rumen are high. Due to hydrophilic properties of hemicellulose, fractional rate of passage of fluid through the rumen decreases because hemicellulose absorbs a greater proportion of fluid and reduces fluid outflow rate. This phenomenon is most likely to occur when high roughage diets are fed.

Van Weyenburg et al. [76] observed higher fluid passage rates in Lucerne hay than in grass hay. Analysis of the hemicellulose content in both feeds showed higher hemicellulose content in grass hay than in lucerne hay. The water holding capacity of the hemicellulose is approximately 260 g water/kg DM for grass hay and 59 g water/kg DM for lucerne hay [76]. This suggests that the greater the hemicellulose content of forages, the greater the amount of water that forage can hold. The greater the quantity of water held by the forage then the lesser the proportion that leaves the rumen, resulting in a decrease in the fractional rate of fluid passing through the rumen. Seemingly, Froetschel and Amos [77] found no correlation between water

holding capacity of digesta and fluid outflow rate, but a positive correlation between water holding capacity and ruminal fluid volume. More evidence of this subject is needed.

Dietary roughage quality affects rates of passage of solid material through the rumen [1]. Rinne et al. [78] found out that clearance of digestible plant cell wall fractions of particulate matter was slower than indigestible fraction of matter. This is perhaps due to sorting of particles in the rumen by stratification [79] and entrapment of digestible material in the floating fibre mat. Within a feed particle, digestible portions of feed retain for longer periods in the rumen and degrade slowly to a high extent, whereas indigestible portions clear from the rumen through passage quickly because of their size and density. Plant particles undergoing fermentation produce gas, hence float and get entrapped in the floating fibre mat restricting their passage out of the rumen. As a result, fractional clearance rate of indigestible part of fibre such as lignin is more rapid than that of digestible fractions such as hemicellulose [80] and may reduce rumen fill [6]. Contrary to this view, Baumont et al. [81] suggested that increases in lignin content of roughage would make it stay much longer in the rumen before being cleared through passage out of the rumen, increasing rumen fill as a result. Baumont et al. [81] was of the view that retention time in the reticulorumen depended on the rate of degradation of the degradable fraction and on the proportion of non-degradable fraction. This suggests that increased proportions of non-degradable fractions slowed down the rate of degradation of the degradable fractions, with overall effects of slowing down passage rate and increasing rumen fill. For microbes to get access to the digestible fractions of fibre, microbes must etch into and remove lignin so as to access these digestible fractions. Hence, as a result of high-lignin content, the rate of degradation is greatly reduced, thus increasing retention time in the reticulorumen. Grasses tend to contain high contents of neutral detergent fibre compared to browse leaves and legumes. Browse leaves are shown to contain much more lignin compared to grasses [82]. Panjaitan et al. [83] reported mean retention times of lignin fraction that were three times greater than those of neutral detergent fibre fraction across four grass species.

Rumen fill is at times described based on fibre (neutral detergent fibre, NDF) fraction [84] on the basis that fermentation and passage rate of neutral detergent fibre through the rumen are slower than of any other dietary constituent. Fibre exerts a greater filling effect in the rumen [6]. Indirect evidence on the effects of NDF content on rumen fill exists. Using sheep fed on alfalfa hay and orchard grass hay, Baumont et al. [85] observed higher dry matter intakes in sheep fed alfalfa hay relative to orchard grass hay, which was attributed to lower NDF content in alfalfa hay. Due to lower NDF content in alfalfa hay than in orchard grass hay, alfalfa had a lower filling effect on the rumen due to rapid rates of fermentation and passage through the rumen [86]. In conclusion, low NDF content is associated with low rumen fill levels, suggesting a positive linear relationship between NDF content (x-axis) and rumen fill (y-axis), which reaches a plateau when rumen capacity cannot increase further with additional increase in NDF content.

Grazing herbivores have an ability to gradually modify rumen volume and increase passage rates in accordance with a reduction in roughage quality [87]. Due to slower passage rates of the digestible fraction, ruminants fed on highly digestible feed may experience maximal rumen fill. Boudon et al. [11] stated that attainment of maximal rumen fill would limit feed

intake in dairy cows grazed on highly digestible rye grass. Also, rumen fill in grazing animals varies greatly from the beginning to the end of a feeding session [11]. On the contrary, Dove [88] suggested a relationship, whereby rumen fill played a major role in regulation of feed intake with decreasing digestibility of a feed. Digestibility is negatively related to lignin content and high lignin content caused slow passage rate in the rumen [78, 88]. This actually suggests that passage rate of solid material was slower for low digestible feeds. Slower passage rates increased rumen fill because feed stays for a much longer time in the rumen. Faverdin et al. [89] demonstrated a more or less similar phenomenon where the overall effect of indigestible feed components resulted in increased rumen fill of approximately 1 kg DM which resulted to a corresponding depression in feed intake of 0.6 kg DM/day. The most probable explanation for this would be a reduction in the rates of clearance of digesta from the rumen, mainly by passage.

3.4. Ambient temperature

As ambient temperature fluctuate during the course of the year due to seasonal changes, or as the day progresses from sunrise to sunset; animals respond to these changes in varied ways and to different extents. Temperatures that might lead to severely altered physiological processes would result in changes in rates of passage of fluid and solid through the rumen. These include temperature ranges above and below the thermo-neutral zone for ruminants [50].

Warm-blooded animals mainly respond to high environmental temperatures by panting, sweating or licking own body, which loses heat from body fluid via evaporation. Increasing plasma volume to increase heat dissipation [90] possibly via radiation may occur. Under extremely high temperatures, animals become reluctant to eat thus reducing dry matter intake [30] to cut down on heat production and heat increment due to feeding. In response to rather low environmental temperatures, warm-blooded animals shiver increasing movements of body parts to generate heat energy internally and generally tend to increase dry matter intake [30].

Low ambient temperatures generally lead to increased rates of fluid and solid passage through the rumen. Kennedy [91] reported a 21% decrease in mean retention time of solid digesta in the rumen as ambient temperature decreased from 21 to 0°C. This increase in the rate of passage may be attributed to occurrence of shivering and increased movements of various body parts [30]. Contraction and expansion of muscles and organs in close association with the rumen may exert pressure on the rumen wall causing it to contract and decrease in size momentarily. Thus, exertion of some pressure on the rumen and its contents may force out rumen fluid and solid from the rumen at a much faster rate. Extents to which such an occurrence affect rates of passage of fluid through the rumen is virtually undocumented and may require further study. Increased rumen movement has been documented at low ambient temperatures [30], probably as a result of movement of organs in close proximity to the rumen. Such movements of the rumen are accompanied by increased power of ruminal wall contraction [30], which may squeeze rumen digesta resulting in it escaping from the rumen at a more rapid rate. Increased dry matter intake on exposure of animals to low ambient temperature [92] is also assumed to have an effect of increasing passage rate of fluid through the rumen due to a push effect.

In extremely high ambient temperatures, slower rates of fluid passage through the rumen are due to a decrease in the pushing effect on the ruminal fluid as a result of low intake. Contrary to that, Chaiyabutr et al. [90] observed that higher ambient temperatures resulted in an increase in the rate of fluid passage through the rumen even when a decrease in feed intake occurred.

Rates of passage of fluid from the rumen increased by almost double from an ambient temperature that is within the thermo-neutral zone of cattle to a temperature slightly above the thermo-neutral zone (**Table 2**). The observed increase in blood and plasma volumes indicated that animals responded to heat stress dissipating heat via evaporation and radiation through the skin thus cooling their bodies using blood water as a medium. Water has a high specific heat capacity with reference to biological systems and hence may be used to dissipate heat [93] in most animals.

The rumen acts as a water reservoir [8]. Water that contributed to an increase in plasma levels may have been from two sources, water intake and rumen, or both. Water may either enter blood through flowing across the ruminal wall [90]; however, proportions that go through this route are minute [94] or diffusion into the blood stream through the intestines [95]. Assuming that water was rapidly mobilised from the intestines into the blood, the capability of the intestines to provide large amounts of water is unlikely. Since the rumen acts as a fluid reserve, it is likely that water would pass from the rumen into the intestines for absorption into the blood. As water from the intestines is lost into the blood, a high water concentration gradient between the rumen and the intestines is created. Suction power for water from the intestines would become high resulting in an increased flow of fluid through the rumen into the intestines [90]. Most studies have reported contradictory results to those of Chaiyabutr et al. [90] on the effect of high temperature on fluid passage rates, thus making this appear as a special adaptation strategy of Swamp buffalo (*Bubalus bubalis*). Further research is needed to prove this theory.

Warren et al. [96] observed increased levels of water intake with increasing ambient temperature. A study by Waybright and Varga [97] showed increased fluid passage rates of up to 64% in water-infused rumen. Tactile stimulation of the distended rumen wall triggers relaxation of the reticulo-omasal orifice and contraction of the rumen wall resulting in rapid flow and passage of fluid and particulate matter through the rumen [98]. Studies by Warren et al.

Environmental temperature	**26°C**	**41°C**
Rate of flow (l/h)	1.82	3.12
Rumen retention time (h)	18.7	13.5
k_1 (per hour)	0.06	0.086
Blood volume (ml/kg)	63.95	68.08
Plasma volume (ml/kg)	47.45	50.83

k_1, fractional passage rate. Adapted from Ref. [90].

Table 2. Effect of heat stress on blood volume, plasma volume and fluid passage rate through the rumen of Swamp buffalo.

[96] stated that mean retention time was directly related to or affected by ambient temperature rather than feed intake as influenced by temperature. Desert species are expected to have faster rates of passage compared to species of similar rumen physiology from temperate regions [28]. The study by Warren et al. [96] used Holstein cattle which are adapted to temperate climates; hence, it is expected that a temperate breed would respond to high ambient temperatures of above 32°C to a great extent. Ruminants that are well adapted to high ambient temperatures in tropical and sub-tropical climates may respond to temperatures of 32°C and above in a different way and probably to a lesser extent when compared to temperate breeds. A change in passage rate as a result of fluctuations in ambient temperature is very high, and the direction of change is unpredictable necessitating more research on the subject. Research needs to focus on the effects of differences in thermal resistance and/or thermal tolerance levels on passage rates in ruminant animals in the tropical regions. These suggest that studies need to consider season and place of study to index ambient temperature when modelling liquid passage rate to take into account the future effects of global warming on digesta passage kinetics. The research needs to consider accounting for the effect of ambient temperature on passage rate.

3.5. Stage of reproductive cycle and physiological state

The reproductive cycle can be subdivided into the lactational and non-lactational period, pregnancy stage, non-pregnancy stage and the number of days in gestation. During the productive cycle, animals undergo structural and functional changes during gestation and lactation [99]. Behavioural changes like loss or gain of appetite and increased or decreased water intake may be observed during these stages [100]. Rate of passage of liquid and solid material through the rumen may be altered by these changes. Gunter et al. [101] showed that rates of particulate and liquid passage through the rumen were higher for pregnant than non-pregnant animals, higher in lactating animals than their non-lactating counterparts, but lower during the late than the early stages in gestation (**Tables 3** and **4**). Helander et al. [102] suggested that different fractional solid and liquid passage rates should be used when formulating diets for pregnant and lactating ruminants.

During pregnancy, nutrient requirements for pregnant animals are higher than for non-pregnant animals [30]. This is due to high demand for protein and energy used for foetal growth [103] and development. Rumen fluid contains dissolved protein [104], short-chain fatty acids [105] and microbial protein. Because of increased demand for the above mentioned nutrients, an increase in rates of passage of fluid through the rumen is observed as a physiological response to meet the increased demand for nutrients in pregnant animals [100].

During the lactation period, there is high demand for water [101, 106], minerals and soluble protein for the process of milk production [30, 106]. All nutrients for milk synthesis are absorbed across foregut walls and small intestines into the blood stream for transportation to the mammary gland. Rumen fluid serves as a water reservoir in ruminants and contains dissolved minerals and soluble proteins [8]. High demand for water in the lower intestines may result in mobilisation of water stored in the rumen. Hence, ruminal fluid passes out of the rumen at a faster rate to meet animal's requirements for water and minerals for milk

Phase	Parameter	Lactating	Non-lactating	Non-pregnant	Pregnant
Liquid	Outflow rate (l/h)	0.7	0.5	0.4	0.5
Liquid	Rate of passage (%/h)	11.1	8.1	10.9	13.9
Liquid	Turnover (h)	9.3	12.7	9.5	7.5
Liquid	Rumen volume (l/kg BW)	0.07	0.08	0.05	0.04
Solid	Rate of passage (%/h)	4.6	4.3	4.9	6.8
Solid	Gut fill (g/kg BW)	5.7	7.7	6.8	4.8
Solid	Mean retention time (h)	26.6	27.9	24.4	18.1

Adapted from Ref. [101].

Table 3. Gut fill levels, and rumen liquid and solid passage rates in pregnant, non-pregnant, lactating, and non-lactating ewes.

	Parameter	Phase	d 102 gestation	d 118 gestation	d 132 gestation
Non-pregnant	Gut fill (g/kg BW)	Solid	5.6	3.8	5.0
Pregnant	Gut fill (g/kg BW)	Solid	6.1	6.6	7.7
	Outflow rate (l/h)	Liquid	0.6	0.4	0.5

Adapted from Ref. [101].

Table 4. Influence of gestation stage on rumen fill levels and fluid outflow rate in ewes.

production. When an animal is non-lactating, there is no demand in water for milk production; thus, the rate of passage of liquid through the rumen is much lower than in lactation. This is in accordance to Chaiyabutr et al.'s [90] theory that increased water demand in the lower gut might result in increased movement of water out of the rumen to meet demand in the lower tract (see Section 3.4). Consequently, the authors [102, 107] observed increases in dry matter intakes of about 20–30% from pregnancy to early lactation, which explains higher rates of liquid and solid passage through the rumen during lactation than during pregnancy [101, 108]. Work on sheep revealed increased rumen fluid volume of 15% during lactation compared to fluid volume at pregnancy [109] supporting the theory of increased water demand during lactation. Contrary to these findings, Hartnell and Satter [110] showed 10%/h higher fluid dilution rates for grazing non-lactating than lactating cows fed silage, suggesting the necessity of more data on the subject. Hence, investigations of effects of interaction between lactation and/or non-lactation period and diet type on dilution rate need to be done.

The rumen and pregnant uterus are in close proximity in the abdominal cavity [109]. It is therefore common sense to assume that as a foetus increases in size there is likelihood that it exerts a pressure on the ruminal wall [76, 99]. This pressure may at least squeeze the rumen thus forcing out some liquid and solid particles with a much greater rate than prior to pregnancy. Increased occupation of abdominal cavity space by growing foetus in pregnant ruminants may have an overall effect depressing total rumen volume. Rumen fill would

be expected to decrease exponentially in pregnant cows as pregnancy progresses. Dairy cows in early lactation have shown increased incapability of consuming enough feed to meet daily requirements for energy. To a certain extent, diminished rumen volume as a result of squeezing from growing foetus causes a reduction in available space for the rumen to expand in anticipation of increased feed intake. Hence, reduction of rumen fill is a result of pregnancy, due to a decrease in rumen volume. Forbes [111] reported an approximate decrease of 0.39 l/l in volume of ruminal contents as pregnancy progressed in sheep fed on hay. However, Kaske and Groth [109] observed increased rumen fill levels from mid pregnancy (60–80 days post conception) to lactation (35–55 days postpartum) with fill levels of 0.946 and 1.444 kg DM, respectively, in ewes. Percentage dry matter content of digesta increased modestly, mean retention times of liquid and small solid digesta reduced by 20–30% at late pregnancy compared to mid pregnancy, with fluid passage rates being approximately three times faster than small solids in sheep [109]. Fluid outflow rate through the rumen increased by 20–36% between late pregnancy and lactation [109]. Generally, rumen fill levels are expected to decrease with an increase in passage rates of solid and liquid digesta. Progressive increments in rumen fill levels in the course from mid pregnancy to lactation were suggested to be due to a gradual reduction in sensitivity of mechanoreceptors on the rumen wall [112]. Such findings may suggest that reticulorumen volumes during various stages of the reproductive cycle may not depend on availability of space in the abdominal cavity alone. They may depend on numerous factors such as diet quality and nervous system response.

Time spent eating and the number of eating sessions were higher during pregnancy than lactation in ewes [102]. Similarly, Kaske and Groth [109] showed a 19% increase in chewing frequency from mid-pregnancy to lactation in sheep. Duration of eating periods and perhaps increase in chewing times may have some effect on rates of liquid and solid passage through the rumen. Oshita et al. [25] showed higher fractional rates of liquid passage through the rumen in non-lactating cows grazed on rangeland (13.95%/h) than those fed fodder ad libitum in stalls (9.4%/h). Animals that graze on rangelands spend more time chewing and eating than those confined to pens [25, 53]. Cows have a greater frequency of rumen contractions during eating than during both rumination and rest [67]. Processes of chewing and rumination stimulated rapid movement of material from the rumen into the reticulum [79] compared to resting. Typical values for frequency of rumen contractions are 1.4/min at rest, 2.3/min during ruminating and 2.8/min during grazing [113] for cattle. It is therefore assumed that frequency of rumen contractions in goats and sheep is not documented. The greater the number of ruminal contractions the greater the fractional rate of liquid and solid passage through the rumen [67]. Thus, animals that spend more time grazing on rangelands have faster fluid and particulate passage rates through the rumen than stall-fed animals. Okine and Mathison [67] concluded that the major determinant of digesta flow through the rumen is a result of reticular contractions. Distension of reticulorumen wall would stimulate an increase in rumen contractions. Fractional passage rate of NDF out of the rumen increased by about 34% as a result of increased rumen contractions [114]. One may tend to wonder the true effect of NDF on rumen fill. Earlier discussions pointed out that high NDF content is associated with increased rumen fill levels.

From an angle associated with reticulorumen contractions, fibre or NDF is a major contributor to increased tactile stimulation of the rumen wall. It may be argued that high levels of NDF

in the rumen would increase the intensity and frequency of rumen contractions through tactile stimulation of the rumen wall. This would result to increased passage of digesta out of the rumen with an overall effect of reducing rumen fill.

So far, a general trend in results showed higher fluid and solid passage rates in lactating than non-lactating ruminants. However, contrary effects have been reported. Oshita et al. [25] observed 10%/h higher fractional passage rates for fluids in non-lactating than lactating cows when fed off silage. These results raise a question on effects of diet and lactation and diet and non-lactation interactions on rates of passage. Further research is needed to cover the gap in knowledge on these observations.

3.6. Particle size and functional specific gravity

Particulate matter is discriminated from moving out of the rumen at two major points in the gut, which are at the dorsal rumen and at the reticulo-omasal orifice [79] because of particle size and functional specific gravity. The likelihood of particles escaping from the rumen is strongly determined by particle size and density [21]. These two factors are inversely related when fermentation has not occurred [115], but in the course of fermentation, Lirette and Milligan [116] observed a negative curvilinear relationship between functional specific gravity and particle size. Various work on effects of particle size and FSG on passage rate have reported similar findings. Allen and Mertens [117] suggested the passage of particulate matter depended on how much particles were present near the reticulo-omasal orifice during the second contraction of the rumen, suggesting that passage rate of solids depended on density. Functional specific gravity of a particle is defined as a physical measure of the weight of a given volume of a particle in the rumen relative to the same volume of fluid in the rumen [8]. The functional specific gravity is determined mainly from the chemical makeup of the lignocellulosic matrix [118]. Lechner-Doll et al. [21] showed a negative correlation between particle density and mean retention time in the rumen. Before fermentation occurs, a solid particle is intact and tends to be heavy (high functional specific gravity) enough to sink to the bottom of the rumen, close to the ventral part of the rumen where its chances of moving out of the rumen through the reticulorumen orifice is increased. So, at this point, movement is only prevented by particle size. Hence, particles tend to have differential passage rate, where it tends to be higher for unfermented particles.

In the course of fermentation after the lag phase (colonisation of feed particles by bacteria), gas is produced from and stays within feed particles [79]. Gas production within particles increases buoyancy of large particles, and as a result, particles tend to float and become entrapped in the floating fibre mat. Probability that these trapped particles are cleared from the rumen through passage is reduced as they would remain trapped until fermentation is completed. Thus, the rate of passage is slow for particles undergoing fermentation. Overall, high fermentation rate may depress the functional specific gravity through increased buoyancy thus reducing the rates of passage. Smith et al. [119] showed that grasses containing higher levels of fermentable organic matter than legumes had much higher retention times in the rumen as a result of increased susceptibility of being trapped in the floating fibre mat. This supports a phenomenon whereby slower passage rates are associated with high

fermentation rates and proportion of degradable matter. Thus, Rinne et al. [78] found that clearance of digestible plant cell wall fractions of particulate matter was slower than indigestible matter. Bayat et al. [120] also showed faster passage rates for indigestible neutral detergent fibre compared to that of potentially degradable neutral detergent fibre of a smaller particle size (**Table 5**).

Although increased reticulorumen contractions have been shown to increase passage rates of both solid and liquid through the rumen, this may directly apply to fermenting solid material because of variable functional specific gravity. The authors [98, 118] suggested that increasing the intensity of rumen contractions actually decreases the rate of passage of particles with low specific gravity from the rumen because contractions propel particles further away from the exit point, the reticulorumen orifice, before it even opens. Inferences on the effect of reticulorumen contractions on passage rate should be specific on which fraction of solid matter and on the value of specific gravity.

Particle size	Parameter	Grass		Red clover	
		Early	**Late**	**Early**	**Late**
		Rumen digesta (kg)			
Large	iNDF	0.88	0.97	1.59	2.36
Large	pdNDF	3.20	3.37	1.83	1.68
Small	iNDF	1.16	1.41	1.36	2.41
Small	pdNDF	2.34	2.61	1.36	1.46
		Mean retention time (h)			
Large	iNDF	28.7	24.3	49.8	37.6
Large	pdNDF	13.9	14.8	13.4	11.0
Small	iNDF	23.8	24.6	29.0	29.8
Small	pdNDF	15.2	14.9	17.9	16.2
		Potentially degradable NDF			
Large	k_p	0.0034	0.0038	0.0041	0.0039
Small	k_p	0.0280	0.0271	0.0242	0.0252
		Indigestible NDF			
Large	k_p	0.0050	0.0062	0.0046	0.0049
Small	k_p	0.0428	0.0424	0.0356	0.0343

iNDF, indigestible neutral detergent fibre; pdNDF, potentially degradable neutral detergent fibre; k_p, fractional passage rate of particulate matter. Adapted from Ref. [120].

Table 5. Effects of particle size and digestibility on mean retention time, rumen fill levels and rates of passage in the rumen.

The theory of the ability of particles to sink (sedimentation) and/or float (stratification) in the rumen resulting in passage out and/or entrapment in the rumen may be true for species of ruminants (grazers) where stratification occurs. There is overwhelming evidence that stratification does not occur in the rumen of browsing ruminants [3]. Passage of particles out of the rumen in browsers is by mass flow, determined by abundance of digesta in the rumen and is normally a function of the occurrence of reticulorumen contractions [4]. Reticulorumen contractions are one of the most important factors that lead to passage of digesta out of the rumen. More studies have to be done to clarify why browsers characterised by lower occurrences of rumen contractions may have faster passage rates of digesta compared to grazers. The authors [21, 121] suggested that lack of stratification was responsible and strongly linked to reduced particle retention times in the rumen of browsing ruminants. It is suggested that particulate matter in browsing ruminants flows out of the rumen at a rate that is proportional to fluid flow rate. It thus remains to be determined how and to what extent passage rates of fluid affect passage of small solids and vice-versa. Thus far, the selectivity factor (SF) is the only proposed measure of the relationship between mean retention times (passage rates) of solid and liquid particles in the rumen (SF = MRT particles ÷ MRT liquid). SF quotient values are used to describe ruminant ecological differences and find application in classification of ruminants into different feeding types [4]. Given that rumen retention time is a function of roughage quality, SF may be used to describe physiological differences in the degree of adaptation of ruminants to different roughage qualities. Nsahlai et al. [122] proposed a relationship that took the form: $kl = (k_p - 0.0018) \div 0.360$. Both these relationships are mathematical in nature and do not give the clear biological relationships between passage rates of the two phases of rumen digesta. Given that both liquid and solid digesta phases exist intermingled together in the rumen, studies need to consider developing passage rate models that can be used to predict passage rates for both phases using one model.

Reduction in size of large particles of feed is a prerequisite for particulate flow out of the rumen via the reticulo-omasal orifice and may be an important determinant of rumen fills [6]. Particle size reduction occurs during rumination or rechewing of previously swallowed feed [91]. The authors [123, 124] showed that resistance to particulate flow through the rumen increases with an increase in particle size. The rate of passage of particulate matter is inversely related to particle size [79]. There is, therefore, a critical size that particle should reach for them to pass out of the rumen via the reticulo-omasal orifice [21]. There are suggestions that critical particle size ranges from 1 to 4 mm [21, 123]. Small dense particles tend to fall into the ventral rumen just close to the reticulorumen orifice [125].

These small particles are capable of passing out of the rumen at the occurrence of the reticular contractions [126] because they would have reached a size that permits passage. Large particles that have a high density are prevented from passing out of the rumen [127] because of sedimentation of these particles at the bottom of the rumen [128]. These particles would still be large and hence are unlikely to pass out of the rumen. The theory of critical particle size as a prerequisite for particulate passage out of the rumen may be questionable because larger particles than this are prevalent in faeces. The authors [129, 130] showed that reticulorumen contractions were accompanied by drastic increases in outflow of solid particles termed

to be large particles (particles greater than 5 mm). McBride et al. [131] argued on how the so-called large particles are prevented from leaving the rumen yet the diameter of the reticulorumen orifice opening of 35 mm [132] is sevenfold greater than the critical particle size. Kaske et al. [128] revealed that when sedimentation was prevented in the rumen of sheep, outflow of 10-mm-sized particles was 40% of the outflow of 1-mm-sized particle, which shows that a great fraction of large particles do leave the rumen. An argument that can be raised is whether or not particle size is an important factor that leads to increased mean retention times in the rumen or it is the effectiveness of the floating mat in entrapment and sedimentation of large particles that determine passage rates to a greater extent than particle size.

Rates and extents to which solid particle size may be reduced depend on fragility of particles. Now, inclusion of particle fragibility as a factor that influences passage rate and ultimately rumen fill opens a new dimension to the current discussion. As noted earlier, high chewing frequencies have an overall effect of increasing passage rates through stimulation of reticular contractions. Chewing also reduces time for particle size reduction ensuring that particles reach a critical size that allows them to pass through the reticulo-omasal orifice swiftly. It can be hypothesised that highly fragile particles pass out of the rumen much faster than less brittle particles. This may be supported by the fact that brittle particles take a much shorter time to undergo particle size reduction, and thus would have a shorter retention time in the floating mat than less fragile particles. This gives more fragile particles a faster passage rate than less fragile particles. Egan and Doyle [80] explained a faster passage rate of indigestible fibre components such as lignin using this phenomenon. Taking a closer look at possible causes of particle fragibility, a contrary effect of fragibility on passage rate is developed. Increased fragility of plant fibre is caused by high lignin content. As a result, degradation rate of high lignin containing particles is reduced, hence more time is required by microbes to colonise and ferment digestible components of fibre. This would result in increased retention times of high lignin particles in the rumen for efficient fermentation. Hence, these particles are likely to be retained for a much longer time in the floating raft. This phenomenon may be aggravated when there are large-sized particles with high lignin content, whereby particles would be restricted by size from flowing out through reticulo-omasal orifice, resulting to reduced passage rates.

4. Are goats different in passage rates compared to other ruminants?

4.1. Background discussion

Generally, rates of passage of solid digesta are greatly dependent on the quality of diets ruminants consume. Botanical and nutritional compositional preference of plant feed sources in ruminants varies greatly. Although goats are classified as intermediate feeders [18], they are selective feeders. Goats demonstrate their botanical wisdom through a mastery of selecting high-quality leafy parts on shrubs, trees and grass stalks that are of higher protein and lower cellulose contents compared to sheep and cattle. This wisdom allows goats to specifically select diets that are able to provide enough net energy and protein to meet their requirements for maintenance for which sheep seem to fail to achieve [133]. This implies that total

tract digestibility and degradation rates of diets eaten by goats should be higher than diets eaten by sheep [36]. Degradation rates of diets consumed by goats were higher than diets consumed by sheep [133], ensuring that goats maintain high intake levels to meet energy requirements (**Table 6**). Goats will spend more time eating per unit lucerne hay compared to sheep, due to their selective feeding behaviour [134]. This mastery in the art of selection of high quality feeds is well documented in goats [36, 134–136] and is proposed to be one of the major reasons why goats have faster digesta passage rates than sheep and cattle. The art of botanical feed selection and preference differs between ruminants, with implications in differential passage rates in ruminants. Consequently, browsing ruminants have shorter mean retention times for liquid and solid digesta in the rumen compared to grazers largely because of increased diet quality.

Feed residues obtained from troughs used for feeding goats had high crude protein and low NDF content compared to those obtained from sheep [36, 134, 137]. These results may be interpreted in two ways. Firstly, it may be that goats select for low crude protein content and high NDF in feeds compared to sheep. However, sheep select plant feed materials of high cell wall content when compared to goats on pasture [133]. Goat selection for diets with low crude protein seems to be a phenomenon common to trough-fed goats. Secondly, the use of residues left behind after feeding and trough feeding of goats and sheep do not give clear results on diet and/or feed selection in these two ruminant species. Differential feeding behaviours occur in trough-fed goats and sheep. When fed from troughs, goats eat feed from top to bottom, whereas sheep eat from bottom to top. High crude protein and low NDF feed particles are finer than low crude protein and high NDF feed particles and are found at the bottom of feed troughs [36]. This implies that goats fed using feed troughs are more likely to consume low crude protein and high NDF diets. Differences in diet selection between goats and sheep fed through feeding troughs warrant more research.

	Proportions (%)					
	I		II		III	
	Goats	**Sheep**	**Goats**	**Sheep**	**Goats**	**Sheep**
Grass	78	80.5	76.5	76.5	80.5	78.5
Shrubs	8	8.5	23.5	23.5	17	19.5
Trees	14	11	0	0	2.5	2
c (per h)	0.038	0.038	0.089	0.068	0.063	0.053
	NDF consumed (g NDF/kg DM)		Digestibility of DM		Digestibility of NDF	
L	662	658	0.495	0.524	0.471	0.521
M	677	671	0.475	0.522	0.466	0.533
S	660	656	0.480	0.493	0.446	0.475

I, April to May; II, May to June; III, June to July; L, long staple length; M, medium staple length; S, short staple length. Adapted from Refs. [36, 133].

Table 6. Botanical and chemical compositional characterisation of diets consumed by goat and sheep.

Botanical variation in diets consumed by goats and other ruminants are wide (**Tables 6** and **7**) and dependent on seasonal availability of different classes and types of feeds in each climatic region. Although predominantly grazers, cattle consumed diets that contained 84 and 48% woody plants in the late wet season and early dry season [139]. Number of plants selected by goats and sheep (25 plants) grazing in a semiarid thornbush savannah were similar, but lower in cattle (10 plants). While total eating time was evenly shared between monocotyledonous and dicotyledonous plants in sheep across all seasons, cattle tend to select monocotyledonous plants (90% of total eating time) and goats consistently selected dicotyledonous plants (82% of total eating time) [136]. Sheep diets contained lower lignin levels in the wet season compared to goats due to selection against browse by sheep [138].

The question on whether goats have faster passage rates than sheep, cattle and other ruminants by virtue that they select less of fibrous plant material is debatable. Generally, goats had faster passage rate than sheep (0.069 vs. 0.033/h when fed as a group; 0.054 vs. 0.029/h when fed individually) when fed formulated diets meeting requirements for maintenance and lactation [140]. Other workers have reported faster passage rates of solid digesta [36, 133, 140–142], slower passage rates [143] and similar passage rates [27] in goats compared to sheep fed on the same diets. Schlecht et al. [142] observed faster passage rates in goats than cattle fed on the same diet (0.042 vs. 0.033/h when fed on bush hay; 0.053 vs. 0.042/h when fed on green feed).

Discussions on differences in passage rates between ruminant feeding types and species that do not consider effects of factors influencing digesta passage rates highlighted here lack descriptive and explanatory power. Given the large number of factors implicated in differential passage rates among goats and other ruminants, a digesta passage rate modelling exercise was used to test the null hypothesis that passage rates in goats are not different from other ruminants (grazers: cattle, sheep, buffalo, antelopes, mouflons, muskoxen, nilgai, blackbucks; browsers: moose, okapi, deer's, dik-dik, duikers; intermediate feeders: goats, anoa, reindeer, gazelles and ibex).

4.2. Methodology

Data were collected from studies that reported at least average values or ranges for body weights of animals used, measured fractional passage rates and/or mean retention times in the reticulo-rumen. A dataset was created bearing passage rates from wild and domesticated ruminants. Factors that affect passage rates were identified in each of these studies and included animal and feed factors. Quantification of factors that affected passage rates are described in [144]. Process models developed as part of this study have been deposited into the Repository of Intelligent Models (REDIM) with accession number PRDA001762 and PRCN001814 for the estimation of solid and liquid passage rate, respectively, as indicated at http://www.redim.org.za/?search=PRDA001762 and http://www.redim.org.za/?search=PRCN001814.

	Crude protein consumed (g/kg DM)				Proportions (%)	
	Cattle	Sheep	Goats		Goats	Sheep
DS	45 ± 5	100 ± 10	125 ± 15	Grass	5.3	14.2
INT	70 ± 10	115 ± 15	125 ± 15	Browse	45.3	40.2
GS	110 ± 10	175 ± 25	195 ± 15	Forbes	44.1	41.5

DS, dry season; INT, intermediate season; GS, green season. Adapted from Refs. [136, 138].

Table 7. Botanical and crude protein of diets consumed by cattle, sheep and goats in different seasons.

4.3. Results

After correcting for variation in 17 (liquid passage) and 23 (solid passage) factors that affect passage rates in the model, predicted solid and liquid passage rates for goats lay near the ideal prediction line and generally embedded with other ruminant feeding types (**Figures 2** and **3**).

This sparse distribution and entanglement of passage rates for goats within that of other ruminants strengthen the view that goats cannot be easily distinguished from other ruminants based on digesta passage rates; so, differences between goats and other ruminants are largely due to variation in diet quality.

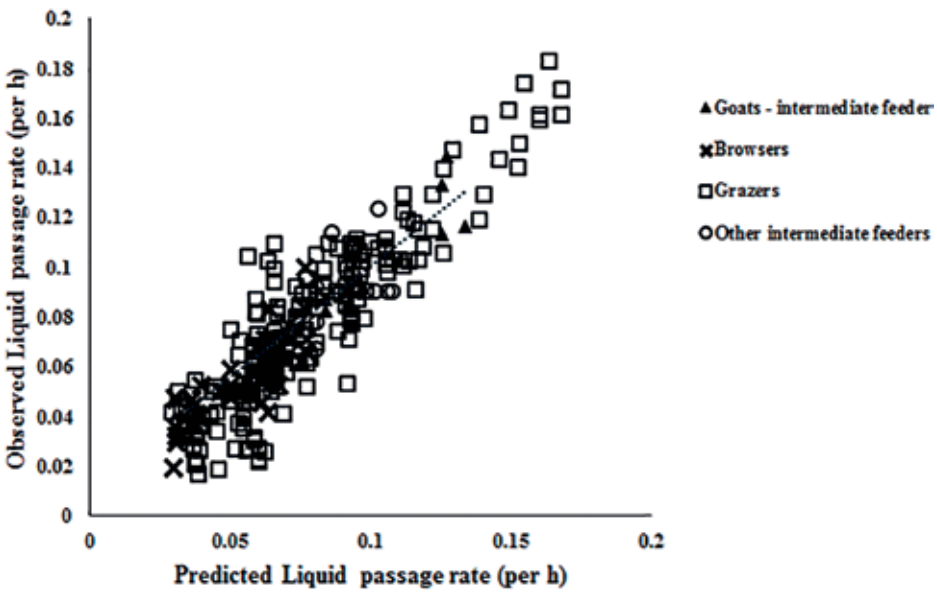

Figure 2. Relationship between observed and predicted liquid passage rates for goats and other ruminant feeding types.

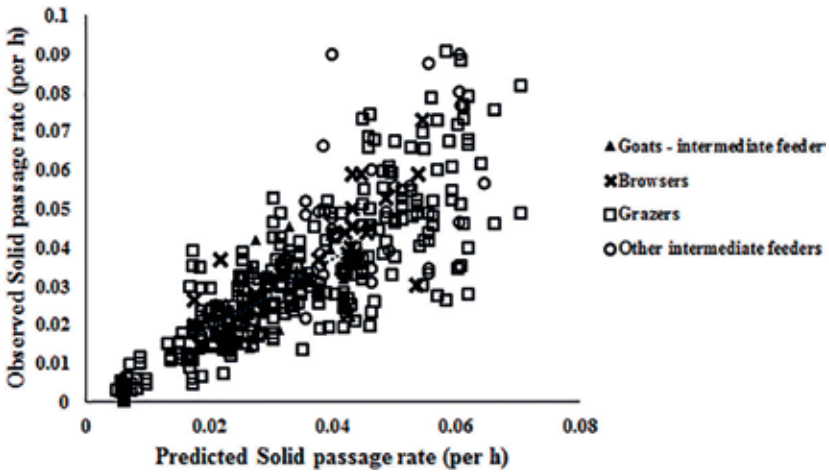

Figure 3. Relationship between observed and predicted solid passage rates for goats and other ruminant feeding types.

5. Summary

Countless factors influence passage rates. Research has not considered effects of various combinations of factors on rates of passage of solid and fluid through the rumen. Mathematical models that seek to accurately predict passage rates, rumen fill levels and ultimately roughage intake should increase understanding of why part of the variation is not explained.

Animal and feed compositional attributes are the major factors to be included into passage rate prediction models. The role of animal physiology in influencing digesta passage rate is critical. Accounting for the influence of various physiological changes in ruminants, feeding level, stage of pregnancy and lactation, and growth in passage rate models can be done by computation of the feeding level based on total net energy requirements relative to net energy requirement for maintenance (animal production level, APL). It is evident that there are still discrepancies on how ambient temperature and particle density (buoyancy) affect the passage rate of digesta in the rumen. Indexing for buoyancy in solid passage rate, prediction models would likely involve determination of the extent of degradability of a particle taking into account the time available for digestion.

6. Conclusion

After correcting for variation in factors that affect solid and liquid passage rates, goats are not different from other ruminants with respect to passage rates suggesting differences between goats and other ruminants are largely due to dietary quality. More studies should be carried to ascertain the dynamics of digesta kinetics after meal termination in goats.

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Chapter 4

Iodine Deficiency in Goats

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Additional information is available at the end of the chapter

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Abstract

Iodine deficiency is a common problem among humans and livestock throughout the world. Prevalence is very high in goats due to less access to soils and browsing habits of goats. It is primarily due to deficiency of iodine in soil, feed, fodder, and water or secondarily due to the presence of goitrogens in diet of animals. Clinical deficiency is characterized by cardinal signs of goiter, whereas subclinical deficiency is difficult to diagnose because clinical signs are not evident. Clinical signs are more prevalent in kids as compared to adults. Diagnosis is on the basis of clinical sign of goiter and estimation of thyroid hormones, the plasma organic iodine level. Milk and urine iodine levels are good indicators of iodine deficiency. Deficiency can be prevented by daily supplementation of iodine and avoiding diets high in goitrogens.

Keywords: iodine, goats, goiter, deficiency

1. Introduction

Iodine is an important micromineral that has a vital role in the synthesis of thyroid hormones like triiodothyronine and thyroxine. These hormones have a role in thermoregulation, increasing cellular respiration and energy generation and have widespread effects on intermediary metabolism, growth, development, reproduction, muscle function, immune defense, and circulation [1].

The synthesis of thyroid hormones takes place in the thyroid gland of all species of animals and also in goats. The size of the gland is approximating 0.20% of bodyweight [2]. It contains the highest concentration of iodine (0.2–5%) of dry weight and in the largest amount (70–80%) of total body iodine.

It is a bilobed structure situated slightly behind the larynx. Right and left lobes of thyroid gland lie lateral to the trachea and are joined by a thin isthmus that passes across the ventral aspect of the trachea [3]. The thyroid gland is a highly vascularized tissue constituted by the functional unit called thyroid follicle. Each follicle has a spherical structure composed of an

outer monolayer of follicular cells surrounding an inner core of colloid, the thyroglobulin-hormone complex, which is the storage reservoir of thyroid hormone. The colloid stored in the lumen is a clear, viscous fluid. The size of the follicles and the height of their cells vary according to the functional state of the gland. The cells may vary from an inactive squamous cell to the highly active, tall columnar cell.

Thyroid hormones (T4, T3, and rT3) immediately on entering the circulation are bound to transport proteins, mainly to thyroxine-binding globulin (TBG), in lesser amounts to thyroxine-binding prealbumin (TBPA), and to albumin. There is a wide spectrum of species variation in hormone binding by serum proteins. TBG is the major binding protein for hormone, but not all species have TBG; however, TBPA is present in all species [4].

The soil in large geographic areas of the world is deficient in iodine. About 29% of the world's population, living in approximately 130 countries, is estimated to live in areas of deficiency.

Iodine deficiency is more prevalent primarily in mountainous regions such as the Himalayas [5], the European Alps, and the Andes, where iodine has been washed away by glaciations and flooding. Iodine deficiency also occurs in lowland regions far from the oceans, such as Central Africa and Eastern Europe. Globally, 2.2 billion people are at risk for iodine deficiency disorders (IDD). Of these persons, 30–70% have goiter and 1–10% have cretinism. The clinical disorders of iodine deficiency tend to be more profound in geographic areas associated with coexisting selenium and vitamin A deficiencies and in regions where goitrogens are fed in diet [6].

Iodine deficiency in large areas of the world is associated with iodine cycle in nature. Iodine occurs in the soil and the sea as iodide. Iodide ions are oxidized by sunlight to elemental iodine which is highly volatile. The concentration of iodide in seawater and air is about 50 μg/l and 0.7 $\mu g/m^3$, respectively. Iodine in atmosphere is returned to the soil by rain and snow which has a concentration in the range of 1.8–8.5 μg/l. The return of iodine is slow and small in amount compared to the original loss, and repeated flooding further decreases iodine in the soil. High rainfall, snow, and floods increase the loss of soil iodine due to melting of glaciers in hilly area due to global warming [7].

Nutritional iodine deficiency in livestock is the leading cause of thyroid gland disorders/goiter. It generally occurs in farm animals wherever human goiter is endemic. Goat is considered as indicator species of iodine deficiency because of browsing habits and less ingestion of soil compared to other grazing animals [3].

2. Material and methods

This chapter is regarding iodine deficiency in goats. It includes the etiology, clinical findings, diagnosis, treatment, and control of iodine deficiency in goats. The authors also include figures of an outbreak of abortion and premature birth of kids with goiter in a flock of goats of Jammu and Kashmir state. Blood samples were collected for hematology, thyroid profile, and estimation of plasma iodine. Urine and water samples were also examined for iodine estimation. Histopathology of thyroid gland was also performed. Affected goats were treated with iodized oils with complete control on occurrence of such condition in future.

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3. Etiology

Iodine deficiency is of two types, that is, primary and secondary or conditional deficiency.

Primary deficiency is an environmental deficiency due to the low level of iodine in soil, water feed, and fodder of animals. A deficiency of iodine in soil and subsequently in fodder crops is the primary reason for iodine deficiency in animals. Soil deficiency may be due to leaching of iodine from surface soil and poor replenishment with airborne iodine [8]. Drinking of groundwater containing iodine less than 2 μg/l leads to iodine deficiency.

In general sandy soils are low in iodine. High clay content and high pH of soil interfere with the iodine uptake by plants growing on such soils.

Iodine content of plant varies with species, strains, climatic and seasonal conditions, and chemical fertilizer supplemented to plants. Cereals, wheat bran, and oil cakes are poor in iodine, whereas straws and green fodders contain marginally adequate content of iodine as per requirement by livestock. Stage of maturity and cutting time significantly affect the iodine content of the fodder. Iodine content of fodder decreases with fall in environment temperature and vice versa [9, 10]. The excessive use of chemical fertilizers like DAP and potash decreases the uptake of iodine from soil; conversely, supplementation of seaweeds in soil will increase the iodine content of soil.

Secondary or conditional deficiency is due to the presence of certain substances present in some plants called goitrogens. It results in iodine deficiency disorders in animals despite of normal iodine intake (1–4 ppm of dry matter), because it interferes with the utilization of dietary iodine or with its metabolism in hormone synthesis. Goitrogens in feed and fodders of animals increase the usual dietary requirement of dairy animals by four to five times.

The presence of goitrogens in diets consisting largely of cruciferous plants like cabbage, *Brassica* spp., peanut, soybean, and yellow turnip contains cyanogenetic glycosides that are goitrogenic because on hydrolysis yields of hydrocyanic acid due to structural damage of the cell wall of plants and further converted to thiocyanates by ruminal microbes. Action of thiocyanates can be overcome by increasing supplementation of dietary iodine. Goitrin (thiooxazolidone) is a thiouracil type of goitrogen present in the seeds of rape, kale, and other *Brassica* spp. It inhibits hormone synthesis, and its action cannot be overcome by extra supplementation of dietary iodine. Linseed meal contains glycoside arachidoside (linamarin) which is converted into thiocyanates in the rumen. Subabool (*Leucaena leucocephala*) contains an alkaloid mimosine (3–6%) which not only inhibits the utilization of iodine but also prevents the availability of iodine from other ingredients in the ration fed to animals. Drinking of grossly bacterial-contaminated water and ingestion of such feedstuffs reported to develop goiter in ruminants. A continued intake of the grass (*Cynodon aethiopicus*) and African pearl millet (*Pennisetum typhoides*) with low iodine and high cyanogenetic glycoside contents may cause goiter in lambs and goats, respectively.

Excess intake of calcium decreases the absorption of iodine from the gastrointestinal tract. High fluoride ingestion is also implicated as one of the important factors for development of goiter in animals. Deficiency of cobalt and thus vitamin B_{12} reported to increase thyroxine levels accompanied by marked hypertrophy and hyperplasia of thyroid gland [11, 12].

Among different breeds of goats, Boer goat of South Africa seems to be more susceptible to develop iodine deficiency due to rapid growth. Indigenous breeds of Himalayan region are more resistant to iodine deficiency than Barbari and Alpine goats. The Angora goat is also reported to be very susceptible to iodine deficiency [3].

4. Pathogenesis

Inadequate iodine in the thyroid gland results in the synthesis of uniodinated inactive prehormone instead of the thyroxine, which stimulates the pituitary gland to secrete thyroid-stimulating hormone (TSH). This commonly results in hyperplasia of the thyroid tissue and considerable enlargement of the gland, that is, goiter (**Figures 1** and **2**).

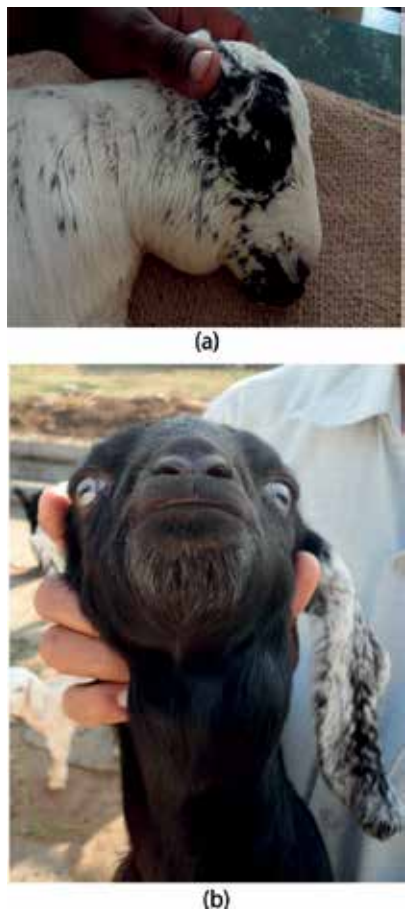

Figure 1. Enlarged thyroid gland/congenital goiter in a kid presented to the University clinics (Courtesy of Dr. R.K. Bhardwaj).

Figure 2. Kid borne with goiter to an apparently normal goat (Courtesy of Dr. R.K. Bhardwaj).

5. Clinical findings

Iodine deficiency occurs in many species of animals and develops different signs in different animals. Goiter is a cardinal sign of iodine deficiency manifested mostly in young ones, that is, kids. The normal thyroid gland is 0.20% of body weight, but it is markedly enlarged up to the size of an orange in iodine-deficient goats [3]. The thyroid gland of goats/kids may be graded as palpable and plum size (+), easily palpable and lemon size (++), and duck egg size, hanging and visible from the distance (+++) (**Figure 3c**). Kids who survive the initial danger period after birth may recover except for partial persistence of goiter (**Figure 1**). The gland may pulsate with the normal arterial pulse and may extend down a greater part of the neck and cause some local edema. Auscultation and palpation of the jugular furrow may reveal the presence of a murmur and thrill (the thyroid thrill) due to the increased arterial blood supply of the glands [12, 13].

Affected kids are born with enlarged thyroid gland or goiter, and enlargement of pituitary gland is also reported [14]. There may be birth of premature kids which are very weak and die within few hours of birth due to severe dyspnea caused by compression of the trachea by enlarged thyroid glands. Majority of new born kids may be hairless/bald (**Figure 3a**) or covered with very fine hair due hypoplasia of hair follicles [15]. Kids may appear dumb or unwilling to suckle the dam [3]. Growth rate of kids is stunted. Fertility of does is affected. Iodine supplementation in goats is found to increase conception, succeed in the first insemination, and reduce abortion rate and dystocia due to goiter [16, 17].

Figure 3. (a) Premature kids aborted with goiter and baldness. (b) Dead kid with goiter. (c) Exposed enlarged thyroid gland (Courtesy of Dr. R.K. Bhardwaj).

6. Diagnosis

Diagnosis of iodine deficiency is based on the history of supplementation of mineral mixture and iodine in the diet of animal and clinical signs like stillbirth, abortion, birth of weak kids, or dead kids with congenital goiter. It is easy to diagnose clinical iodine deficiency. The subclinical deficiency is of more importance as it is difficult to diagnose and goes unnoticed. Subclinical iodine-deficient animals show few or no clinical signs, but production, growth rate, and fertility are affected.

Thyroid hormone (triiodothyronine and thyroxine) assay is used to confirm iodine deficiency or hypothyroidism in goats. Normal range of thyroxine is 3.3–7.0 μg/dl and is decreased in iodine deficiency [3]. Triiodothyronine is an active form of thyroid hormone at the cellular level which needs to be converted from thyroxine to triiodothyronine by selenium-containing enzyme deiodinase. Deficiency of selenium may result in increase in the level of thyroxine and decrease in triiodothyronine and elevated plasma cholesterol.

Estimation of protein-bound (organic) iodine in blood is highly sensitive for diagnosis of iodine deficiency. Low protein-bound (organic) iodine below 8.1 μg/dl is suggestive of iodine deficiency [3].

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The rate of excretion of iodine in milk and urine can provide useful diagnostic criteria in simple iodine deficiency since iodine intake is positively correlated with urinary iodine excretion. The milk iodine level of sheep is 80 μg/l and in the deficiency level is below 8 μg/l. Similarly, lower normal limit of iodine in urine is 50 μg/l.

Normal thyroid gland contains acini lined by low cuboidal epithelium filled with colloid, whereas in goiter, it is replaced by tall columnar epithelium, papillary in foldings, and reduced colloid. A hyperplasic goiter is characterized by enlarged gland with much colloid also known as colloid goiter when dietary supplementation of iodine is made to goats. Iodine content of the thyroid gland is reduced (**Figure 4a–c**). Subclinical iodine deficiency can be diagnosed histopathologically by hyperplasia of the thyroid epithelium with grossly normal size of the thyroid gland [3].

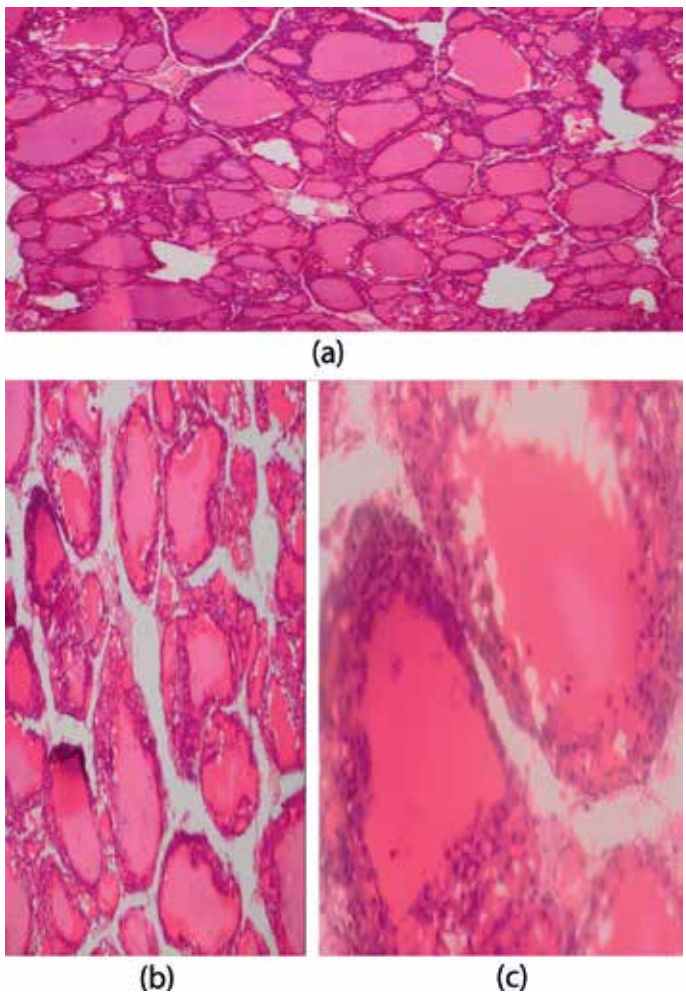

Figure 4. (a) Histopathology of enlarged thyroid gland at 10×, (b) 40×, and (c) 100× showing various sizes of thyroid follicle with colloid and hyperplasia of the epithelium of thyroid follicles (Courtesy of Dr. R.K. Bhardwaj).

7. Treatment and control

The actual dietary iodine requirement is 0.8 mg/kg dry matter for lactating goats and 0.5 mg/kg dry matter for the rest of the flock. When goats are fed cruciferous plants, iodine requirement is approximately 2 mg/kg dry matter to prevent iodine deficiency [19].

Iodine deficiency goiter is treated or prevented by supplementation of iodine to the goats especially to pregnant does in the form of iodized salts. The recommended iodine content of salt is 0.0190%; it should be supplemented to livestock as 2% in concentrates or 0.5% of the total dry matter intake. The daily iodized salt requirement for goats is 4.5 g for adults and 2–2.5 g for kids [18].

Oral daily supplementation of 130 mg potassium iodide or application of 1 ml of tincture of iodine weekly on the back during pregnancy successfully prevented goiter in goats.

The author has treated outbreak of stillbirth in goats with 28 g of potassium iodide in 1 l of distilled water and 10 ml drench to each doe checked abortion in flock.

Prophylactic injection of 375 mg of iodized oil before servicing of goats prevented relapse of stillbirth and congenital goiter in goats.

In general goiter can be prevented by avoiding goitrogenic diet and forages especially during gestation period and regular supplementation of iodine in the diet of goats.

8. Conclusions

Iodine deficiency is a most common problem in goats all over the world due to browsing habits of goats and ingestion of plants containing goitrogens. Deficiency of iodine and development of goiter in goats are suggestive of iodine deficiency in the area. It can be treated by supplementation of iodine in oral or injectable before servicing and avoiding goitrogens containing feed during gestation period.

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Reproduction

Chapter 5

Reproduction in Goats

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Alejandro Sergio del Bosque González and
Hugo Bernal Barragán

Additional information is available at the end of the chapter

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Abstract

Reproductive activity of the goat begins when the females reach puberty, which happens at 5 months of age. The ovarian or estrous cycle is the period between two consecutive estrus. It is also the time that lasts the development of the follicle in the ovary, until rupture occurs and ovulation takes place, which coincides with the appearance of estrus. This chapter will describe the physiological and endocrinological bases of estrus in the goat. Likewise, factors affecting the presence of estrus and ovulation will be described. At another point, synchronization of estrus and ovulation, factors affecting the presence of estrus and external symptoms of estrus, will be described. To achieve synchronization of estrus or induction of ovulation within or outside the breeding season, it may be necessary to manage light hours, male effect, and/or use of hormones. The importance of artificial insemination is described, as well as the current situation of this technique worldwide. Currently, the techniques of artificial insemination in goats have been limited worldwide, due to the lack of resources of producers and trained technicians. The techniques of artificial insemination with estrous synchronization programs and ovulation with current research results will be described.

Keywords: goats reproduction, estrual cycle, estrous synchronization, artificial insemination

1. Introduction

Since its domestication, the goat has been characterized by being seasonal polyestrous, that is, during certain times of the year, it reproduces naturally. This characteristic varies mainly according to the hours of daylight (photoperiod), rasse, and nutrition [1]. It is mentioned that

the more goat is exploited at latitudes more distant from the equator, the breeding season will be shorter [2]. However, in latitudes nearer from the equator, the presence of estrus will depend on the availability of nutrients and the environment. On average, the goat's estrus cycle is 21 days, and the high frequency of short estrual cycle is characteristic and tends to occur at the beginning of the reproductive season and in young animals [1, 2]. The average duration of standing estrus is 36 hours but can range from 24 to 48 hours depending on age, breed, season, and presence of a male [3].

2. Folliculogenesis

In mammals, germ cells originate from the extraembryonic endoderm and migrate by amoeboid movement into the coelomic cavity, to reach the urogenital mesodermal crest. Subsequently, the germ cell is transformed into oogonium, which should populate the gonad by mitotic processes. At the end of mitosis, the oogonium enters the meiotic cycle until prophase I, where it acquires the primary oocyte state [4]. Therefore, folliculogenesis begins when the primordial follicle is formed, due to the union of the primary oocyte and granulosa cells. In primordial follicles, the primary oocytes leave their state of latency spontaneously and continue to other phases of growth during which the differentiation and proliferation of the oocyte coexist with the surrounding cells and by the effect of the growth factors synthesized in the microenvironment ovarian; all these events are independent of the gonadotropins [5]. Different types of follicles are present during folliculogenesis. Primordial follicle: This follicle begins the process of follicular growth and maturation, to guarantee increasingly mature units that can lead the ovary toward ovulation or atresia. Primary follicle: It is characterized by a significant change occurring when the flattened cells surrounding the oocyte increase in size into a more cuboidal form, grow in diameter, and consolidate as a structure with oocytes having a diameter greater than 22.12 mm and a layer of 25–40 cuboidal cells called granulosa cells. It also increases the volume of the oocyte and the formation of the zona pellucida. Secondary follicle: The transition to this stage depends on the FSH stimulus. At this stage, granulosa cells develop the ability to synthesize growth factors and steroids [5].

3. Physiology and endocrinology of the estrual cycle of the goat

The ovarian cycle is classically divided into two phases: follicular phase and luteal phase (**Figure 1**). The follicular phase corresponds to the wave of follicular development that will provide the ovulatory follicle and involves the maturation of follicles that are dependent on the gonadotropins until ovulation [6]. During the follicular phase, FSH secreted by the anterior pituitary stimulates follicular growth. A cohort of antral follicles, which are gonadotropin dependent and with a diameter of 2–3 mm, is recruited, and the follicles enter their

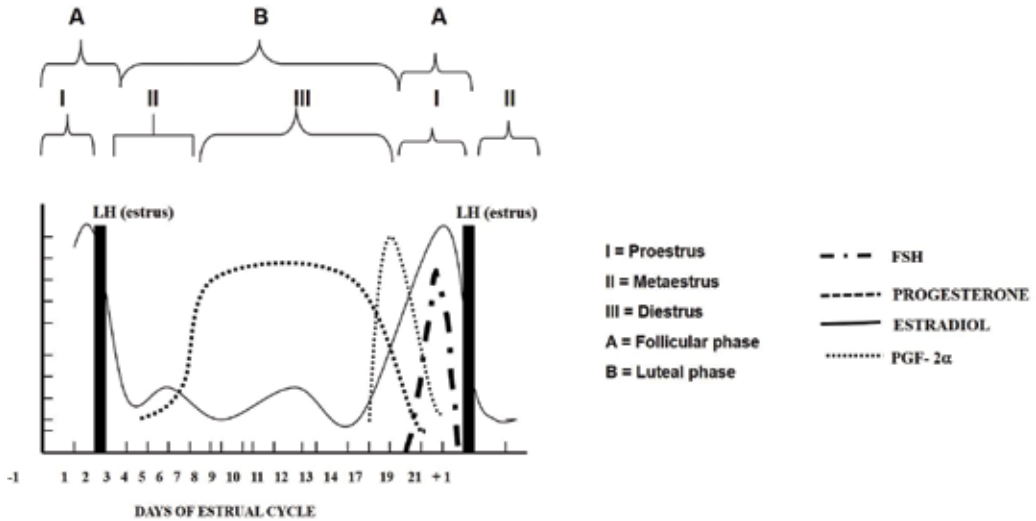

Figure 1. Schematic representation of the estrual cycle of the goat (modified by Fatet et al. [3]).

terminal phase of growth [7]. Only two to three of these follicles reach a size of 4 mm in diameter and are selected to enter the dominance phase. Under the influence of LH, the follicles reach the preovulatory stage (6–9 mm), while the subordinate follicles degenerate (follicular atresia). The increase in the peripheral concentrations of estradiol 17β causes a positive feedback effect on the hypophysis-gonadotropin axis, due to the follicular growth inducing the goat's estrus behavior [8]. The consequent increase in gonadotropin-releasing hormone (GnRH) secretion induces an increase in the preovulatory LH peak, which will induce ovulation between 20 and 26 hours later, and finally the luteinization of follicular cells will occur. The beginning of the follicular phase, before the estrual behavior is observed, is known as proestrus. The estrus phase includes the events of the sexual behavior of the goat until ovulation. Consequently, a response to estrus and ovulation will mainly depend on the time of pregnancy and nutrition. Whereas providing the goat with short energy supplementation can increase the rate of ovulation. During the estrus cycle, the ovaries undergo a series of morphological (follicular recruitment and growth), biochemical (follicle maturation), and physiological (endocrine regulation) changes, which lead to ovulation. These cyclic changes that take place in the gonads are known as ovarian cycles. For follicular growth, it develops in the form of waves throughout the cycle (**Figure 1**). A follicular wave is characterized by the sequence of three gonadotropin-dependent events: recruitment, selection, and dominance. When performing repeated ultrasound studies, one can mention and/or suggest that there are between two and six waves of follicular development during the estrus cycle; in goats, there are usually three–four waves. The last follicular wave is the one that is going to give rise to the ovulatory follicle. When double ovulations occur, they are due to follicles derived from the same wave but two consecutive follicular waves [8, 9].

Also, the luteal phase begins when the corpus luteum is formed after follicle luteinization (duration 16 days). During this phase, LH is released pulsatile and, its frequency is negatively correlated with progesterone. Now, progesterone has a negative feedback effect on LH. Luteolysis begins around day 16–17 of the estrus cycle, releasing uterine prostaglandins, influenced by oxytocin. With the above, the concentration of progesterone decreases, causing a strong increase in the frequency of pulses of LH and its amplitude, which causes ovulation [10].

3.1. Extern symptom of the estrus

The external symptoms of estrus that can be mentioned as important are goats move the tail, increase vocalizations, decrease appetite, mount between them, increase urine excretion, inflammation of the vulva, and discharge of vaginal mucus.

4. Estrous synchronization and induction of ovulation in goats

Globally, the goat was one of the first ruminants to be domesticated more than 10,000 years in Asia. It has been one of the most useful species to man as a supplier of meat, milk, skin, and fibers for families. By 2014, there was a world population of 1011 million heads of goats. A goat has always been considered a family subsistence animal; however, the interest of developed countries to organic food changed the way to exploit it more technically. Fortunately, there has been a change in this attitude, and more recently producers in developed countries have become interested in exploiting goats for their attributes, such as adaptation to adverse climatic conditions, increased meat production, and early slaughter age [11, 12].

At the beginning of the twenty-first century, one of the reproductive tools that has had great impetus in research has been the synchronization of estrus. This technique aims to concentrate the estrus of the goats at different times of the year. Synchronization of estrus involves the development of a luteal phase by means of exogenous hormones (devices with natural or synthetic progesterone) for a specific period and not exceeding the luteal phase of the normal goat cycle [13].

With this technology, farmers can use more efficiently complementary techniques for reproductive management, including artificial insemination (AI) and multiple ovulation and embryo transfer (MOET), such that genetic material is more easily obtained or transferred domestically and internationally [14]. Exogenous hormones are used to modify the physiological chain of events involved in the sexual cycle, while the nonhormonal methods of estrous synchronization involve the use of light control or exposure to a male [15]. In the doe, the window of opportunity is generally greater during the luteal phase, which is of longer duration and more responsive to manipulation. It is essential that any estrous synchronization technique should not only establish synchrony but also ensure reasonable levels of fertility in the synchronized cycle [15].

4.1. Current status of estrous synchronization programs in goats

In goats, estrous synchronization protocols are currently based on the use of vaginal devices (sponges) impregnated with 20–40 mg of fluorogestone acetate or 50–60 mg of

medroxyprogesterone. In the 1980s, controlled internal drug releasing (CIDR) began to be used, which is an inert silicone elastomer containing 0.3 g of natural progesterone (P4) [16]. These devices are inserted intravaginally for a period of 5–14 days (see **Figure 2**) to create a luteal phase and then accompany it with a luteolytic agent, as well as the application of a hormone that synchronizes ovulation (equine chorionic gonadotropin (eCG), estradiol benzoate (EB), gonadotropin-releasing hormone (GnRH)), which have been applied at the time, 24 and 36 hours to remove the device, respectively [17, 18].

4.1.1. Prostaglandin-based synchronization

Prostaglandin F2α (PGF2α) and its analogues have also been used to synchronize estrus by controlling luteal function since PGF2α was discovered to have a luteolytic effect in sheep. During the estrous cycle, PGF2α is secreted by the nonpregnant uterus to 16 days after estrus [5]. Administration of PGF2α after removal of a CIDR mimics the secretion of PGF2α by the uterus, causing lysis of the CL and the onset of a new follicular phase [18]. Administration of PGF2α is effective from approximately d 3 to d 14 of the estrous cycle in sheep [19]. Analogues of prostaglandins can also induce luteolysis and are often more cost-effective. The effectiveness of PGF2α is limited to the active period of cyclicity in small ruminants. The lack of ovulation of the follicle during seasonal anestrous causes a lack of luteal development [19]. A single administration of PGF2α can induce luteolysis, and two PGF2α injections at an interval of 10–12 days have been used to synchronize estrus (see **Figure 3**). These treatments can be used on cycling goats only, limiting its application during the extended periods with nonfunctional corpora lutea [20].

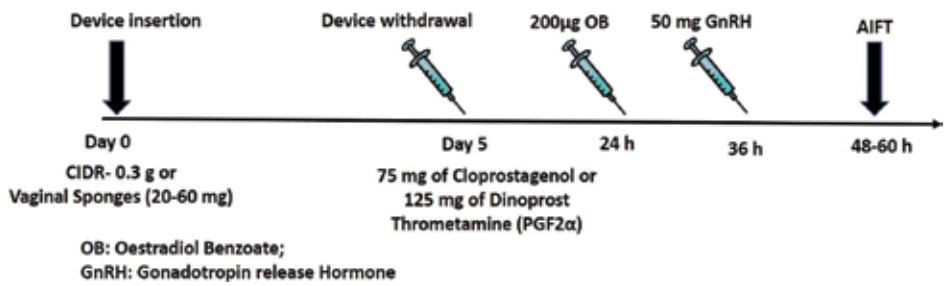

Figure 2. Short protocol at 5 days and ovulation synchronization with OB or GnRH.

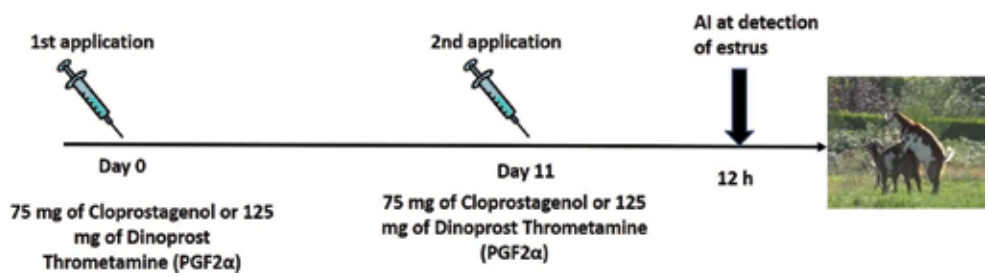

Figure 3. Protocol of synchronization of estrus with prostaglandins within the reproductive season.

Because of the use of PFG2α in the synchronization of estrus within the reproductive season, up to 70% of estrus goats have been obtained. These results are lower than those reported when using different devices with progesterone source [21]. On the other hand, in temperate and subtropical regions, the use of the male effect is a tool that is increasingly being used to avoid using hormones, with a 60% effectiveness in the presence of estrus and ovulation in the first 5 days after the introduction of males. In goat herds, it is important to emphasize that the effect of nutrition plays a preponderant role in the synchronization programs of estrus and ovulation using hormones and male effect or a combination of both within a reproductive program to use AI [22].

4.1.2. Progesterone-based synchronization

The synchronization of estrus in goats by medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA) sponges has been accomplished in many advanced countries in the world [22]. Most commonly, progestogen-/progesterone-impregnated vaginal devices and subcutaneous progestogen implants followed by an injection of equine chorionic gonadotropin ((eCG), formerly PMSG), were used for estrous synchronization [23]. However, a prolonged progestagen treatment is also associated to reduced fertility (related to a subluteal serum progesterone concentration at the end of treatment) [24]. This acts by promoting the growing and persistence of the dominant follicle which causes detrimental effects on fertility. Reduced fertility in prolonged progestagen-treated females may be also related to an impairment of sperm transport in the genital tract [23, 24].

Intravaginal sponges impregnated with progestogens have been extensively used in sheep and goats to control estrus and ovulation during the breeding and non-breeding season. One of the main problems associated with controlled breeding is the estimation of the time and degree of estrous response. Thus, if one can predetermine the time from withdrawal to onset of estrus, the need for estrous detection could be reduced or even eliminated. In goats, fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP) sponges have been found to be equally effective in estrous synchronization (see **Figure 4**) [25].

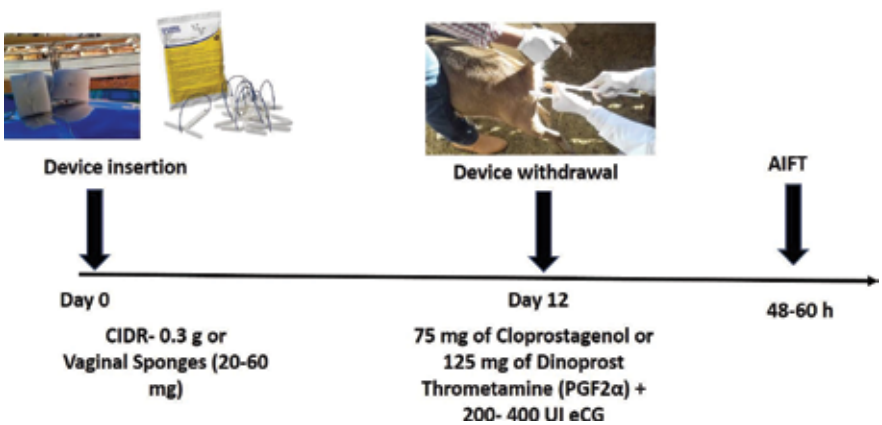

Figure 4. Normal protocol of estrous synchronization.

4.1.2.1. Combined CIDR and PGF synchronization

Research protocols for CIDR inserts have been focused on short-term (5–7 days) and long-term (12–19 days) length in small ruminants [26]. One of the benefits to short-term progesterone protocols is the ability to synchronize females in a short period. This can be beneficial to producers in planning timed AI or ET programs. Short-term protocols typically combine the use of progesterone with multiple follicle-controlling hormones, such as follicle stimulating/ovulation inducing, a combination of equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG), and PGF2α [26]. Using multiple hormonal controls in short-term synchronization protocols gives an increased ability to control luteal and follicular dynamics (see **Figure 4**) [10]. Previous research indicates that serum progesterone concentrations are maintained at an increased level when compared with a long-term progesterone insert protocol [27]. The label recommended protocol for CIDR insert in ewes is 5 days and has been proven to induce ewes and does exhibit estrus during active cyclicity and during anestrous (see **Figure 5**) [1]. Concerns with short-term CIDR protocols include inconsistency in estrus response and increased interval to estrus [27]. Estrus cannot be precisely predicted, and the interval from CIDR removal to estrus can range from 35 to 70 **subsistence** ours [25]. Long-term synchronization protocols in sheep and goats have proven to result in shorter intervals from CIDR removal to estrus when compared with short-term protocols [27].

CIDR devices offer important advantages. Firstly, they contain low natural doses of progesterone [27]. Moreover, unlike intravaginal sponges, CIDRs do not absorb or obstruct drainage of vaginal secretions, resulting in less foul-smelling discharge upon removal. Finally, these devices also induce earlier and more compact synchronization and have a better retention rate during treatment [28]. However, CIDRs are expensive when the benefit/cost ratio is evaluated and compared to vaginal sponges. For this reason, the reuse of CIDR up to 3 times has been expanded in sheep as in goats to reduce the costs of estrous synchronization programs and therefore use more artificial insemination in both species [29].

4.1.3. Male effect

The "male effect" has been a subject of research for nearly 75 years, which began to be published in the first scientific articles in sheep (1944). The male effect is defined as an interaction between

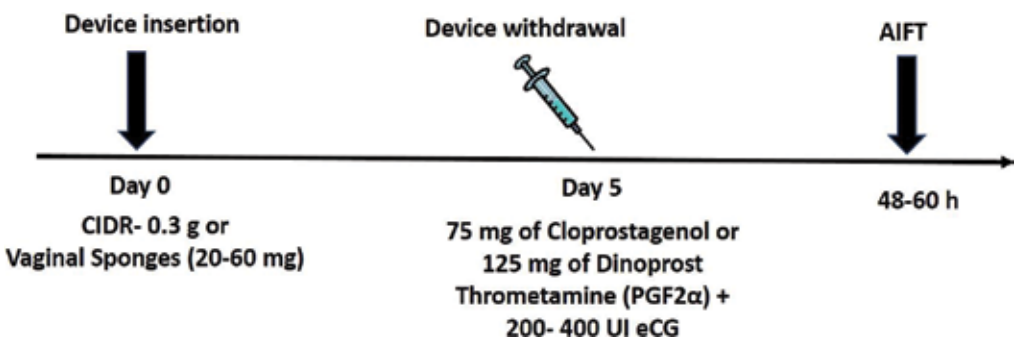

Figure 5. Short protocol of 5 days and artificial insemination at fixed time (AIFT).

a male and a group of females at the time of starting a mating. Physiologically, the contact of a group of goats in anestrous with males induces an increase in the frequency of LH, which gives rise to a preovulatory peak and consequently ovulation before 48 hours (see **Figure 6**). Also, it has been clearly established that the first ovulatory cycle is sometimes accompanied by estrus, although the first estrus is detected until the second ovulation (7–14 days) after the introduction of the males [29, 30]. The recent concept of production described as the "clean, green, and ethical" method seeks to find natural alternatives to achieve the same objective of improved reproductive rates by natural means [31]. However, the male effect should be accompanied by good management practices, animal health and especially nutrition. Recent studies have shown that goats that present subnutrition at the time of introducing the male (energy deficit) present a delayed response up to 7 days to present the first estrus with ovulation compared to goats that have covered the energy requirements during the breeding season. Furthermore, the male response will have a better impact on goats that have a body condition below 2 compared to goats that have a body condition above 3. Within this context, the male effect on dairy goats has also been evaluated, where the presence of estrus occurs later compared to the breeds of meat, where the period of breeding is extended by 31 days more [31]. The presence of bucks creates olfactory, behavioral, and visual stimuli which in an increase in the secretion of luteinizing hormone which stimulates folliculogenesis and ovulation [10]. Although it was initially believed that pheromones played a pivotal role in the male effect, subsequent work has proved that visual and behavioral stimuli are equally as important as the smell [31].

In recent years Delgadillo and Martin [2] published and confirmed that many of the dogmas related to the male effect are not true. For example, they concluded that in goats it is not necessary to fully shear the males, even to the extent that the wind is directed in the herd. On the other hand, the sheeps recognize the new sheep and respond to them without any problem when they join with the sheep in the herd. Likewise, they report that the male effect is minimal in cyclic goats. As it was also mentioned that after the introduction of the male, it should remain permanently in contact with the goats; it is now confirmed that with only 4 hours of contact, this phenomenon occurs. However, in sheep that present a deep seasonal anestrous, they respond to the male effect that is sexually active. To achieve this, a treatment based on a light program (photoperiod) + melatonin implants can be provided, or in bucks, the application

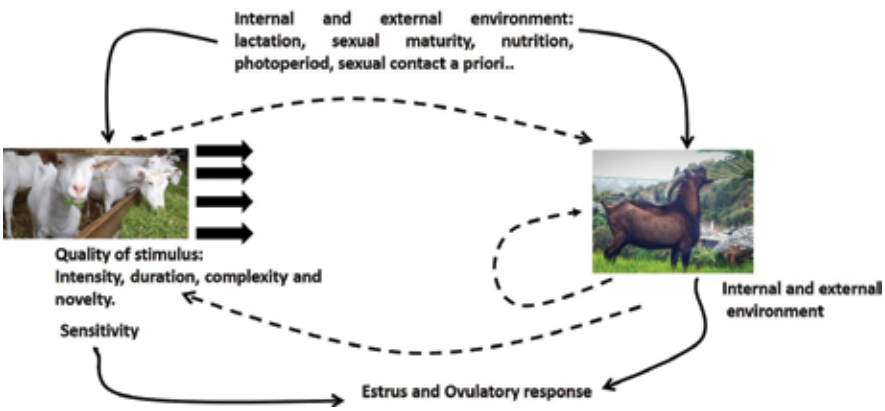

Figure 6. Schematic representation of "male effect," modified from Delgadillo et al. [1].

of testosterone 1 month before male introduction (10 mg/day 3/3 weeks). Likewise, for the goat to respond to this phenomenon, it is necessary the complete contact of both sexes and not only by the smell of the male [31].

4.1.4. Synchronization and induction of ovulation

The use of eCG after progestogen exposure to promote ovulatory follicular growth and ovulation is inquired as repeated eCG treatment is known to affect pregnancy rate in goats [31]. This detrimental effect is associated with an immunogenic response induced by repeated eCG treatment, which is frequent in goats under intensive breeding programs [24]. It can also be used to synchronize ovulation, administering 50 mg of GnRH 36 hours after the removal of the device [31] or 200 μg of estradiol benzoate given 24 hours after the short-term protocol (see **Figure 2**) [32].

Estrous synchronization protocols have been improved to more frequently use fixed-time artificial insemination as well as more effectively implementing third-generation reproductive techniques. These are oocyte production in vitro, cytoplasmic sperm injection, nuclear somatic cell transfer, and pronuclear microinjection of zygotes. At present, additional research is required to lead to an economical and efficient synchronization program to establish efficient protocols for controlled breeding, massive use AI, and embryo transfer by nonsurgical methods [32].

4.1.5. Factors affecting estrous synchronization programs

Various factors affecting synchronization program have been reported, but the genetic and environmental factors (animal breed, photoperiod, temperature, etc.) were shown to be of primary importance in most of the studies [9–11, 33]. Controversial synchronization estrus results (from 40 to 100%) have been determined in dairy goats with artificial insemination by several authors [19, 20, 26]. In most of the experiments, different factors (season, breed, age and body condition score of the animals, estrous synchronization protocol, time and number of AI, and the breeding technology) have been shown as a cause for varying results [33].

4.1.6. Nutrition and body condition

Numerous studies confirm that nutritional management significantly affects goat breeding. When the goats are to be reproduced, one factor that negatively affects the response to the presence of estrus and ovulation rate is body condition. Thus, recent studies have shown that when goats have a body condition below 2, ovulation rate is reduced by up to 16% compared to goats with body condition above 3. In addition, goats with low body condition present a shorter breeding season and estrus cycles of abnormal duration [33]. However, research carried out in recent years shows strong results that, when supplementing goats with low body condition (2), have a higher rate of ovulation (37.5%). Studies carried out in goat grazing confirm that the goats lose their body condition in one unit or more; they present a smaller and more prolonged "response" to the male effect, besides a poor rate of ovulation and of births. Physiologically, in goats, the follicular population is very sensitive to the entry of nutrients, so that folliculogenesis and ovulatory rate can present a favorable response through the supplementation, being monitored the nutritional status through the body condition [34]. In addition, deficient nutrition affects ovulatory cycles, where underfed goats exhibit increased

sensitivity of the pituitary to negative feedback of estradiol, which causes an inhibition of GnRH release and therefore LH. Likewise, in sheep, it has been found that the feeding level determines the live weight and the body condition, presenting a static, dynamic, or acute effect of the nutrition on the ovulatory rate, which depends on the observed changes in live weight and body condition. The "acute effect" is the one that has been given more attention in current research to promote significant changes in follicular population and ovulation rate, without the need to present changes in live weight and body condition [34]. For the above, "flushing" overfeeding strategies have been used, which consist of increasing the energy or protein levels of the goats before and during the breeding, to positively affect the rate of ovulation and prolificacy. Alternatively, it is possible to maintain this nutritional practice 10–15 days after the breeding to contribute to the implantation of the embryo, reducing the early embryonic mortality. Recent studies confirm that goats with a body condition less than 3 respond better than others with greater body condition. Also, it is defined that the changes in the ovulatory rate that receive high levels of feeding positively affect a glucose increase at the cellular level. This implies that glucose participates in the control of ovarian function, where the levels are regulated by insulin, which plays an important role in follicular growth in goats [35].

However, assessment of body condition is a simple indicator of body fat reserves, which can be used by the goat in periods with high energy demand, stress, or undernutrition. Likewise, body condition values have been determined from 1 to 5, where 1 is skinny and 5 is obese. It has been evaluated that the goats must maintain a body condition of 3 so that the reproduction is not affected negatively; here, the visual aspect of the goat would be the backbone is not prominent, ribs are barely discernible, and an even layer of fat covers them. Intercostal spaces are felt using pressure [35].

Dairy goats with excessive fat reserves or over-condition at kidding may have a greater risk of lower milk yield and of increased health and reproductive disorders (see **Figure 1**), such as dystocia and fatty liver. According to Ref. [35], animals with extremely good body condition tend not to respond to flushing.

Reproductive seasonality limits the reproductive efficiency in goat production systems. This seasonal reproductive pattern evolved in goats in a manner that time of parturition and lactation coincides with season of the greatest feed availability and favorable temperatures. In domestic goats, reproductive season starts between the summer and fall and ends between the winter and spring, depending on both breed and geographic latitude [36].

5. Artificial insemination

Artificial insemination (AI) is an important technology for improving animal production. Through consistent use of AI, herd genetics can be advanced at a rapid rate. While AI is a technology that enables the dissemination of selected male genetics, embryo transfer (ET) is a technology that enables the dissemination of selected female genetics, and, by using ET, frozen embryos can be moved around the world at significantly reduced costs compared to movement of adult animals [37]. Utilization of either AI or ET requires careful doe management and is most effective when these technologies are used in conjunction with estrus or

ovulation synchronization. Artificial insemination (AI) involves collection of semen from a buck and transfer of the semen to the reproductive tract of the doe. Does can be inseminated either with fresh semen or with commercially available frozen semen [38].

Other authors mention that AI allows producers to use superior bucks to dramatically improve performance of their herd [38]. However, the rewards of AI depend on sound management. Artificial insemination in goats is more difficult than it is in cattle because of the small size of the animal and the complex anatomy of the cervix, making insemination into the uterus difficult. Advantages of the AI are the following:

1. Genetic improvement via buck
2. Easy transport and introduction of genetic material at the global level
3. Extended semen conservation of genetically superior chives
4. Control of diseases transmitted by natural mountain (up to certain point)
5. Lower inventory of bucks in the herd

The success of AI is dependent on:

1. The appropriate timing of insemination in relation to estrus and ovulation
2. The ability to efficiently collect and cryopreserve (freeze) sperm from quality bucks
3. The seasonality of goat's reproduction

5.1. Techniques of artificial insemination

5.1.1. Artificial insemination transcervical

The cervix of the doe has four tightly closed, cartilaginous rings that provide structure to the cervix and, along with cervical mucus, form a protective physical barrier against the entry of foreign particles. To achieve the highest pregnancy rates for AI, semen must be deposited into the uterine body or into each of the uterine horns. Deposition of semen into the uterus requires that all four cervical rings must be passed during the insemination procedure. The small size of the doe's reproductive tract, particularly for nulliparous (virgin) or young primiparous (once kidded) does, in addition to the tightness of the cervical rings and their typical lack of alignment, can make passing the insemination rod during transcervical AI a challenging task. However, several methods for transcervical insemination have been developed and are available [38], some of which are similar to procedures described for nonsurgical embryo transfer in goats [38].

5.1.2. Standard AI method (tube speculum)

The simplest transcervical AI method involves the use of a tubelike speculum and a standard French-style insemination gun. The speculum, with a detachable light, is inserted into the vaginal vault of the doe and used to visualize the external cervical which is the entry point

into the cervical channel [39]. Frozen semen is available in 1/4cc or 1/2cc straws and must be appropriately thawed prior to use. Once the semen straw is prepared and placed into the insemination gun, a clean sheath is overlaid to protect the semen and reduce cross contamination between does. Sheaths can have either standard (rounded) or apex (pointed) ends that can aid in achieving deeper penetration of the cervix [40]. The insemination gun is introduced through the speculum, and the inseminator attempts to pass the insemination gun through the cervix and deposit the semen into the uterine body. Following insemination, the gun and speculum are removed and the speculum disinfected between does. The single-use AI gun sheath is disposed appropriately. The major advantage of the standard method is that it is a simple and easily mastered technique that is reasonably effective with older, multiparous does (see **Photo 1**). The major disadvantage of this technique is that it is difficult to pass the insemination gun through the small cervix of a young doe or through the cervix if it is highly convoluted. In many cases, the use of the standard technique results in deposition of the semen in the cervix if all the cervical rings cannot be passed. Under controlled conditions, pregnancy rates following the use of the standard technique are low, typically in the range of 20–30% [41–44].

5.1.3. Deep cornual (uterine) insemination (catheter-within-catheter) method

In 2005, the development of a novel method for transcervical insemination of goats has been reported. In their method, semen is deposited deep into the uterine horn (cornua) by means of a catheter-within-catheter technique. This technique relies on the use of a soft, small diameter pediatric urinary catheter stiffened with an insemination gun stylet to enter

Photo 1. Student of agronomy practicing the transcervical artificial insemination in goats.

the uterine body and individual uterine horn. To facilitate passage through the cervix, the doe's hindquarters are raised, and a Pozzi Tenaculum Forceps are used to grasp the cervix and align the cervical rings. Once the catheter is positioned into the uterine horn, the stylet is removed, and a small diameter insemination tubing is threaded through the urinary catheter and used to deposit fresh or frozen-thawed semen into the upper portion of the uterine horn. The urinary catheter is then repositioned into the opposite uterine horn, and the second half of the semen sample is deposited deep into that horn to complete the insemination. With trained technicians, the entire procedure takes about 5–10 min [41] and does not involve any surgical entry or anesthesia of the doe. Furthermore, pregnancy rates following deep cornual insemination were greater than those for laparoscopic insemination in their study. In a subsequent study, pregnancy rates using ovulation synchronization with TAI of a single dose of frozen semen were 58% and kidding rates similar at 53%. These pregnancy rates are comparable to those obtained for beef cattle for first-service insemination after TAI using frozen semen [45].

5.1.4. Artificial insemination by laparoscopy

Currently, artificial insemination techniques in goats and sheep have been limited globally due, among other factors, to the lack of economic resources and trained personnel. Currently, for every 10 cows that are inseminated, only 2 goats and 3 sheeps are inseminated. This low percentage of inseminated goats has caused that the genetic progress is limited in the caprine species. This technique is focused on the integration of high genetic quality flocks that allow them to use high-value semen and that can be used preliminarily in the programs of embryo transfer and in vitro fertilization [45]. One way of evaluating bucks is through their daughters. The fastest way is to use AI to have daughters in different flocks. To achieve this, it is necessary for producers to use AI to propagate genes from different bucks in their herds. Among the techniques of AI that have been used to increase the rate of conception is the technique of laparoscopy, which consists of the deposition of the semen directly inside the uterine horns, avoiding the natural barrier of the cervix (see **Photo 2**). With this technique, pregnancy rates of 80% with diluted fresh semen and 50–80% with frozen semen are achieved. First, the goat is given a water and food diet for 12 hours to reduce the contents of the bladder and rumen. This facilitates the location of the uterus and prevents regurgitation of ruminal contents. The ability of the inseminator will be the time it takes to deposit the semen in both uterine horns [45]. A highly trained technician can inseminate the goat in 2 minutes. Before initiating endoscopy, the goat is anesthetized locally with 5 ml (20 mg) 2% lidocaine hydrochloride subcutaneously (see **Photo 2**). Take special care to avoid injuring the blood vessels when injecting the anesthesia (see **Photo 3**). Local anesthesia aims to relax the goat and not present ruminant contractions and can visualize the uterine horns. Once the ventral cavity is pierced and in the direction of the nipples of the udder (10 cm of the nipples), the endoscope is inserted (see **Photo 3**). The cavity is inflated with CO_2 to facilitate the localization and manipulation of the uterus. The insemination gun is inserted through the second puncture and inserted into the wall of the uterus into the lumen releasing the semen (see **Photo 3**). Usually, the semen is deposited in both uterine horns to achieve good results of pregnancy. Normally, both uterine horns are inseminated with the Aspic pistol

Photo 2. Observation and manipulation of the uterine horns to carry out the technique of artificial insemination by laparoscopy.

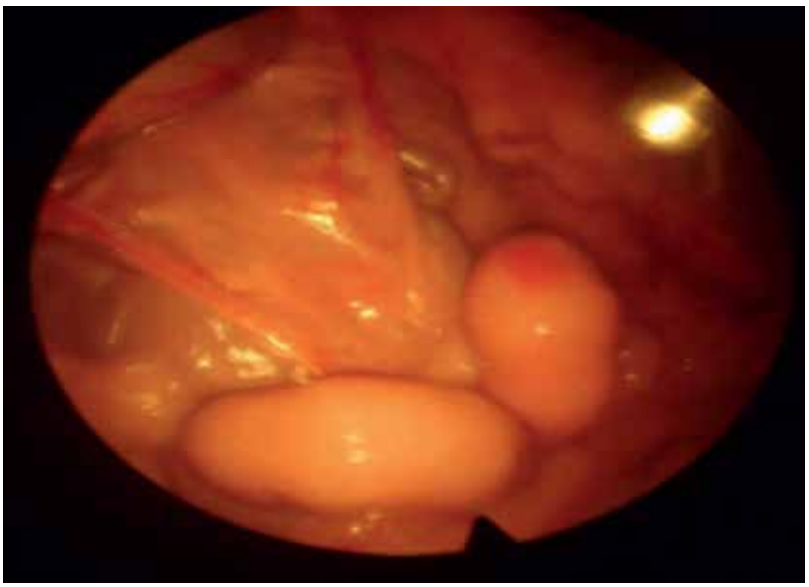

Photo 3. Observation of uterine horns in a goat through laparoscopy with 12 hours of water diet.

(IVM), or the Robertson Pipette from Minitube. The objective is to inseminate in the middle part of both uterine horns, depositing half of the dose in each one of them. The technician must take between 2 and 3 min to inseminate the goat. When fresh semen is used, pregnancy percentages are reached above 80. With frozen semen, percentages are reached between 50 and 80% pregnancy.

Once the artificial insemination is performed, the endoscope is removed, and a commercial disinfectant is placed in the incisions that were made. The amount of sperm deposited per dose varies from 40 to 100 million. The results of this AI technique will depend on other factors, including goats' body condition, insemination time, race, year time, and synchronization protocol, among other factors.

6. Conclusions

Goat production globally has increased in recent years. Therefore, good reproductive strategies are required that are feasible to be applied by producers, such as the synchronization of estrus and ovulation, because it is required to have a more intense selection pressure via bucks (artificial insemination) as goats (embryo transfer and in vitro fertilization).

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Chapter 6

Recent Advances in Research on the Hormone INSL3 in Male Goats

Tetsuya Kohsaka, Siqin, Itaru Minagawa and Hiroshi Sasada

Additional information is available at the end of the chapter

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Abstract

Insulin-like factor 3 (INSL3), previously called relaxin-like factor (RLF), is essential for testis descent during fetal development and has been implicated in the testicular and sperm functions in adult males. However, similar functions in ruminants remain largely unknown. This chapter will cover recent advancement in our understanding of INSL3 in goats. First, testicular Leydig cells were the sole source of INSL3, with INSL3 expression increasing during development. Second, INSL3 was constitutively secreted as a B–C–A single-chain structure with full biological activity. Third, secreted INSL3 was transported into the seminiferous compartments, where its receptor RXFP2 was expressed on germ cells, thus suggesting that the intratesticular INSL3 hormone-receptor system operates in germ cells. Fourth, functional RXFP2 enabling INSL3 to bind was also identified in the spermatozoa and suggested the existence of the extratesticular INSL3 hormone-receptor system in the spermatozoa. Interestingly, percentages of INSL3-binding spermatozoa were significantly reduced in the semen of subfertile bulls compared to that of fertile bulls, suggesting the potential of this system to diagnose fertility in breeding sires. These fascinating findings will give a new perspective in physiological and/or therapeutic actions of INSL3 on male reproductive processes in domestic ruminants, including goats.

Keywords: insulin-like factor 3 (INSL3), RXFP2, expression, structure, function, testis, spermatozoa, goat

1. Introduction

Fertilizable sperm production is regulated by the organized actions of germ cell types and stage-specific gene products and is supported by the complex interplay of many different molecules, including endocrine and paracrine signaling. Of these, insulin-like factor 3

(INSL3), previously called relaxin-like factor (RLF), is a novel member of the insulin/relaxin gene family, and is produced by both fetal and adult testes [1]. INSL3 is essential for fetal testis, and has been implicated in the testicular and sperm functions in adult males [2]; however, similar functions in ruminants remain largely unknown. Exploring the function of INSL3 in goats is especially intriguing, because goats are useful pilot animal for studying reproductive physiology in ruminants because of their fecundity and precocious sexual development [3] and other advantageous traits, such as small body size, calm behavior, and ease of handling. Here, we provide an overview of the recent progress in research on INSL3 in male goats.

2. Insulin-like factor 3 (INSL3)

The relaxin family peptides consist of seven distinct gene products, which include relaxin (RLN), H1-RLN, RLN-3, INSL3, INSL4, INSL5, and INSL6 (**Table 1**), of which INSL3, like RLN, has been extensively reviewed elsewhere [4] and is expressed almost exclusively both in male and female gonads. INSL3 binds with high affinity to and activates its specific receptor, relaxin family peptide receptor 2 (RXFP2), originally called leucine-rich G protein-coupled receptor 8 (LGR8) [5, 6]. INSL3 plays an essential role in testis descent during fetal development by acting on the gubernaculum via RXFP2, as revealed by mouse models targeting genes encoding either Insl3 or Rxfp2 [7]. After birth, INSL3 possibly plays a role for maintaining spermatogenesis by functioning as a germ cell survival/anti-apoptotic factor and as a germ cell proliferation factor in some species [8–10].

2.1. A short summary of research methods

Before moving on to the main subject, we will briefly introduce a short summary of materials and methods that we actually used in this chapter. First, peptide antibody for INSL3 was generated and subsequently used for immunological approaches such as western blotting

Relaxin family peptides		**Receptors**
Name	**Nomenclature**	
Relaxin	RLN	RXFP1
H1-relaxin	H1-RLN	RXFP1
Relaxin 3	RLN3	RXFP3
Insulin-like peptide 3	INSL3	RXFP2
Insulin-like peptide 4	INSL4	?
Insulin-like peptide 5	INSL5	RXFP4
Insulin-like peptide 6	INSL6	?

The data were modified from Ivell et al. [4].

Table 1. The relaxin family peptides and their receptors.

and immunolocalization. Then, INSL3 from goat testes was purified using a series of chromatography steps and the native conformation was examined by tandem MS (MS/MS) analysis. Biological activity of purified INSL3 was examined by cell-based assay based on the cAMP in INSL3 receptor RXFP2-expressing HEK-293 cells. Additionally, the secretory pathway of INSL3 was examined by immunoelectron microscopy. Next, we identified cell types expressing its receptor RXFP2 using both laser capture microdissection and immunostaining with RXFP2-specific antibody and by characterizing its developmental expression pattern and specificity of INSL3 binding. Moreover, we examined the functional RXFP2 protein in the spermatozoa using *in situ* INSL3 ligand binding assay.

2.2. Testicular Leydig cells are the sole source of INSL3 in male goats, with INSL3 expression increasing during sexual development

2.2.1. Generation of an anti-INSL3 peptide antibody

As in other species, *INSL3* transcripts are detected only in the testis in the mature male goat [11]. However, no studies have yet been carried out to identify whether *INSL3* mRNA is translated into INSL3 protein in the goat testis. There are so many commercially available antibodies for INSL3, but none of these antibodies cross-react with goats (http://www.antibodies-online.com). Thus, the first step toward elucidating the possible physiological role of INSL3 in goats is to produce its specific antibody. Based on the cDNA sequence [12], we generated an anti-INSL3 peptide antibody (RLF-A-Ab808) in New Zealand White rabbits against the synthetic peptides of 15 amino-acid residues of the A domain, which shared 100% amino acid homology with boar, bovine, sheep, and goat *INSL3* cDNAs [11]. This peptide antibody was shown by western blotting to recognize the ~12-kDa proINSL3 in the testicular extracts and the ~16-Da recombinant INSL3, which was expressed in *Escherichia coli* by using a construct that expresses the His-tagged proINSL3 sequence containing the B–C–A domain inserted into the pCold-I vector (**Figure 1A**). Furthermore, Ouchterlony gel diffusion test found that one precipitin band between the antibody and testicular extracts of goats and boars or recombinant INSL3 was formed, joined at their ends and fused (**Figure 1B**), indicating that antigens from boars and goats are immunologically identical and are perfectly cross-reactive with antibody. Additionally, the antibody did not cross-react with RLN, which is closely related to INSL3.

2.2.2. Source and expression dynamics of INSL3

As revealed by *in situ* hybridization, *INSL3* mRNA is expressed in the Leydig cells that are well known as steroid hormone-producing cells (**Figure 2**), which is consistent with previous study [12]. However, it is unclear whether *INSL3* mRNA was translated into the protein therein. Using the INSL3-specific antibody, INSL3 protein was identified immunohistochemically in the Leydig cells (**Figure 2**), with INSL3 expression increasing during sexual development [11]. Therefore, it is reasonable to draw the conclusion that testicular Leydig cells are the sole source of *INSL3* mRNA and protein in male goats, which is consistent with the findings that INSL3 is expressed by Leydig cells of both fetal and adult testes in a number of species [1].

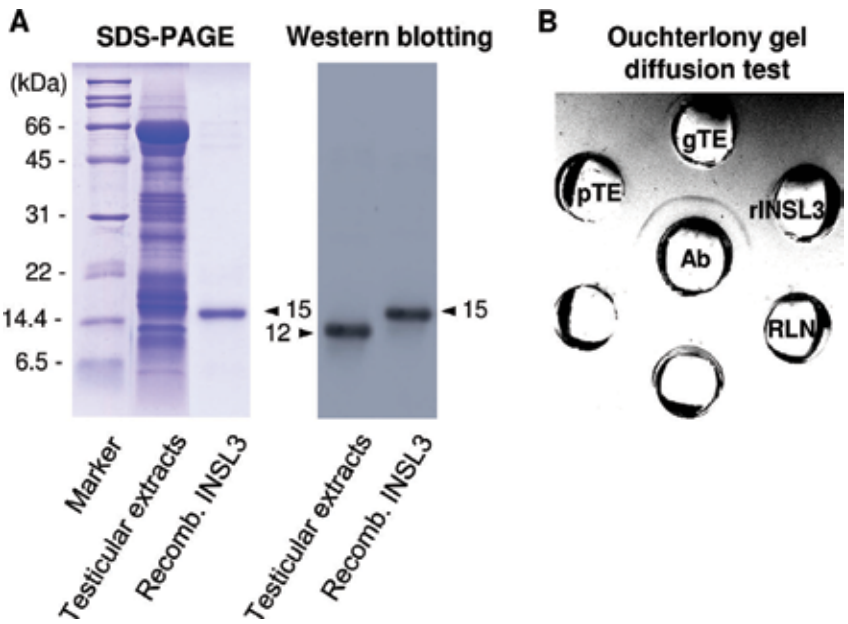

Figure 1. Specificity of anti-INSL3 peptide antibody. (A) SDS-PAGE and western blot analysis of goat testicular extract and recombinant (recomb.) INSL3. (B) Ouchterlony gel diffusion test showing precipitin reactions between the antibody and testicular extracts or recombinant INSL3 (rINSL3). pTE, pig testicular extracts; gTE, goat testicular extracts; RLN, porcine relaxin. SDS-PAGE and western blotting data are unpublished, while Ouchterlony data are derived from Siqin et al. [11].

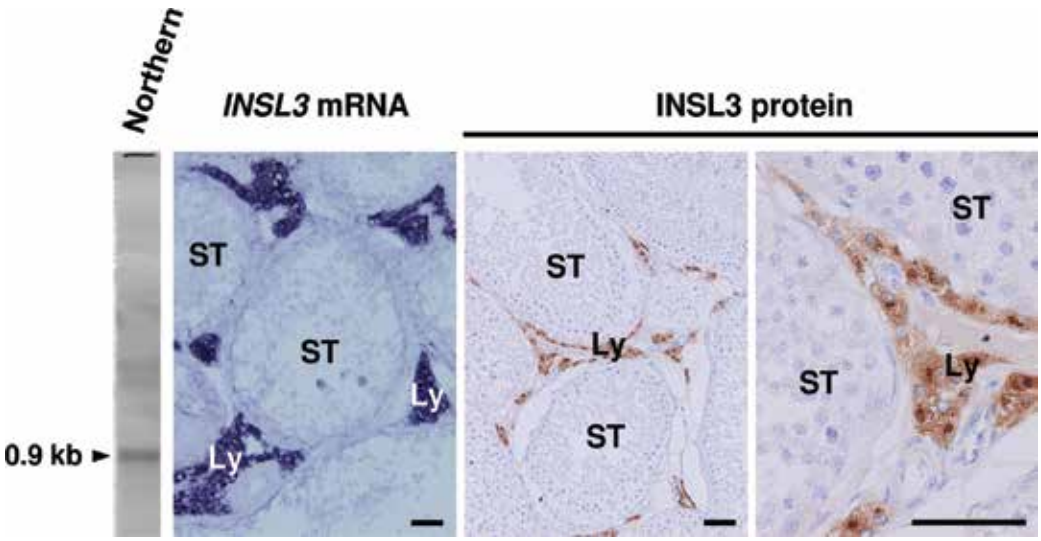

Figure 2. INSL3 expression in the goat testis. *INSL3* mRNA and protein were expressed in the Leydig cells. Northern, northern blotting showing 0.9 kb *INSL3* mRNA in the testis; Ly, Leydig cells; ST, seminiferous tubules. Bars = 50 μm. *INSL3* mRNA data are unpublished, while the protein data are based on Siqin et al. [11].

In common with circulating INSL3, *INSL3* mRNA and protein expression in the testis of rodents and pigs have been reported to be upregulated coincidently with pubertal development [13–15]. In goats, the dynamics of INSL3 production in the testis are fundamentally consistent with those of other species. INSL3 protein was detected as a specific band of ~12-kDa on western blotting of the goat testis (**Figure 3A**), and its signal was high at 1 month after birth, decreased at prepubertal stage (3 months), and then increased markedly at puberty (4 months), remaining at that level through adulthood (**Figure 3B**) [11]. The expression level of *INSL3* gene followed a similar pattern to that of the INSL3 protein [16]. The relatively high expression of INSL3 at 1 month of age might be attributed to the presence of residual fetal

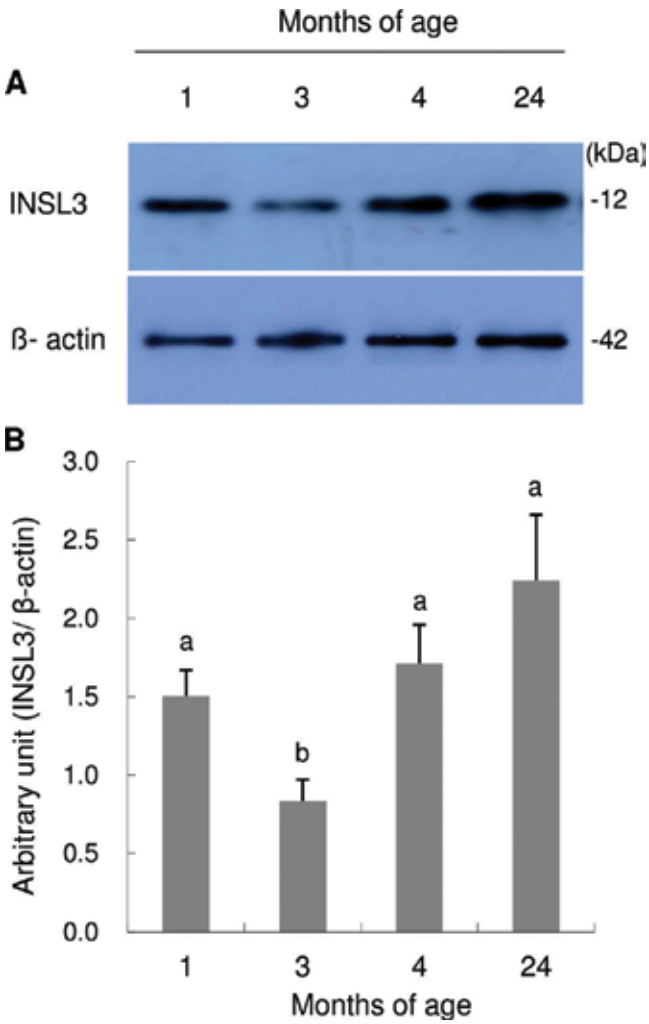

Figure 3. Expression profile of INSL3 protein in the goat testis during development. (A) A representative western blot analysis. (B) Relative level of INSL3 expression. Data were derived from Siqin et al. [11].

Leydig cells, which often persist in the testis at early postnatal stages [17] and express high levels of *INSL3* mRNA and protein similar to those of adult Leydig cells, as observed in rats [18].

As in the case of other species, INSL3 expression in goats appears to correspond fundamentally to the developmental status of Leydig cells. With the establishment of a functional hypothalamic-pituitary-gonadal (HPG) axis during puberty, goat Leydig cells appear to be most responsive to LH at puberty as characterized by a dramatic increase in Leydig cell number and cell diameters [16]. In addition, in other species, INSL3 expression in Leydig cells has been reported to be induced under the long-term effects of LH/hCG and is not acutely regulated by LH/hCG and other hormones influencing Leydig cell differentiation, such as insulin-like growth factor1 (IGF1) [19, 20].

2.3. Goat INSL3 is constitutively secreted from testicular Leydig cells as a B–C–A single-chain structure with full biological activity

2.3.1. Structure of INSL3

Unlike other peptides from the relaxin/insulin family, very little is known about the native conformation of INSL3. INSL3 is predicted from the cDNA sequence to be biosynthesized as a precursor protein (pro-INSL3) containing A- and B-domains connected by a C-domain and is assumed to undergo proteolytic processing to remove the C-domain peptide. The precursor then matures to an A–B heterodimer linked by two disulfide bonds to form an active hormone, as do the other members of this family of peptides (**Figure 4**) [1]. This prediction has been supported by the findings that a synthetic INSL3 peptide, which consists of an A–B heterodimer with site-specific sequential disulfide bonds in some species, such as humans [21] and rats [22], stimulates cAMP production by binding to its receptor, RXFP2 [5]. Furthermore, this is corroborated by a report that native INSL3 exists as an A–B heterodimer when isolated from bovine testis [23].

However, recent study concerning native INSL3 purified from boar testes demonstrated for the first time that the native INSL3 exists as a monomer comprising three domains, B–C–A, with site-specific disulfide bonds and full biological activity [24]. Therefore, there appears to be not only a bovine form of native INSL3 that exists as an A–B heterodimer [23], but also a porcine form that exists as a B–C–A single chain [24]. In fact, the molecular mass of INSL3 detected by western blot analysis in tissue extracts from several species, including goats [11], corresponds to that of pro- INSL3 (B–C–A single-chain form) as deduced from their cDNA sequences. Hence, it is worth proving whether native INSL3 of other species, including goats, exists as a B–C–A single chain that corresponds to a porcine model of native INSL3 but not the bovine model.

When purified the protein using a series of chromatography steps and examined the native conformation by MS/MS (tandem MS) analysis, the native goat INSL3 is isolated as a 12-kDa protein with a B–C–A single-chain structure, in which the C-domain does not undergo proteolytic processing (**Figure 5A**) [25]. This is consistent with the structural features of porcine native INSL3 comprising a B–C–A single-chain form [24] but quite different from those of native bovine INSL3 [23] in that C-domain does not undergo processing. Clarification of

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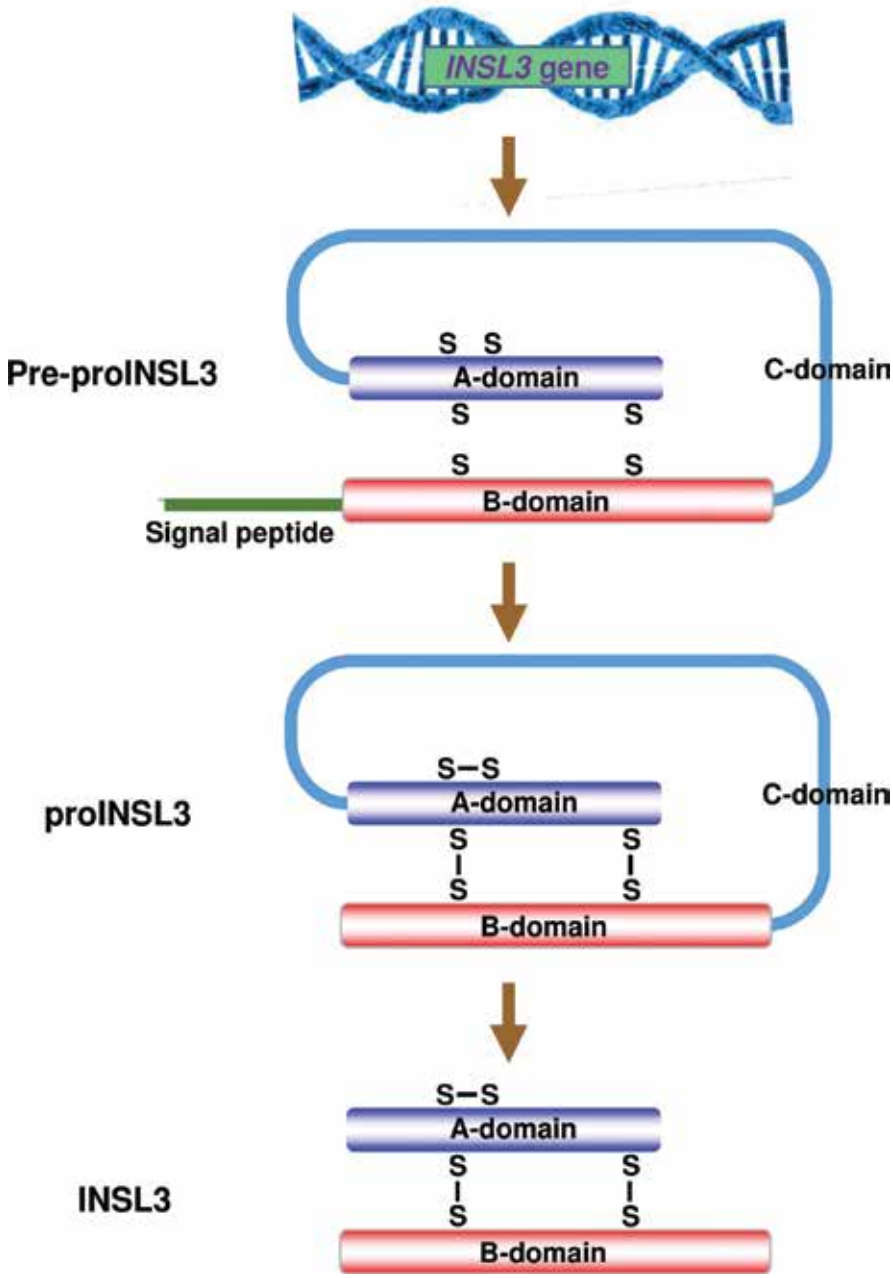

Figure 4. Predicted biosynthetic process of INSL3. INSL3 is predicted from the cDNA sequence to be biosynthesized as a precursor protein (prepro-INSL3) containing: (1) a signal peptide that presumably permits the nascent protein access to the rough endoplasmic reticulum (RER) before being excised; and (2) A- and B-domains connected by a C-domain. After cleavage of the signal peptide in the lumen of the RER, pro-INSL3 is assumed to undergo proteolytic processing to remove the C-domain peptide and then to mature to an A–B heterodimer linked by two disulfide bonds to form an active hormone like the other members of this group of peptides. Predicted biosynthetic process of INSL3 is based on Ivell and Bathgate [1].

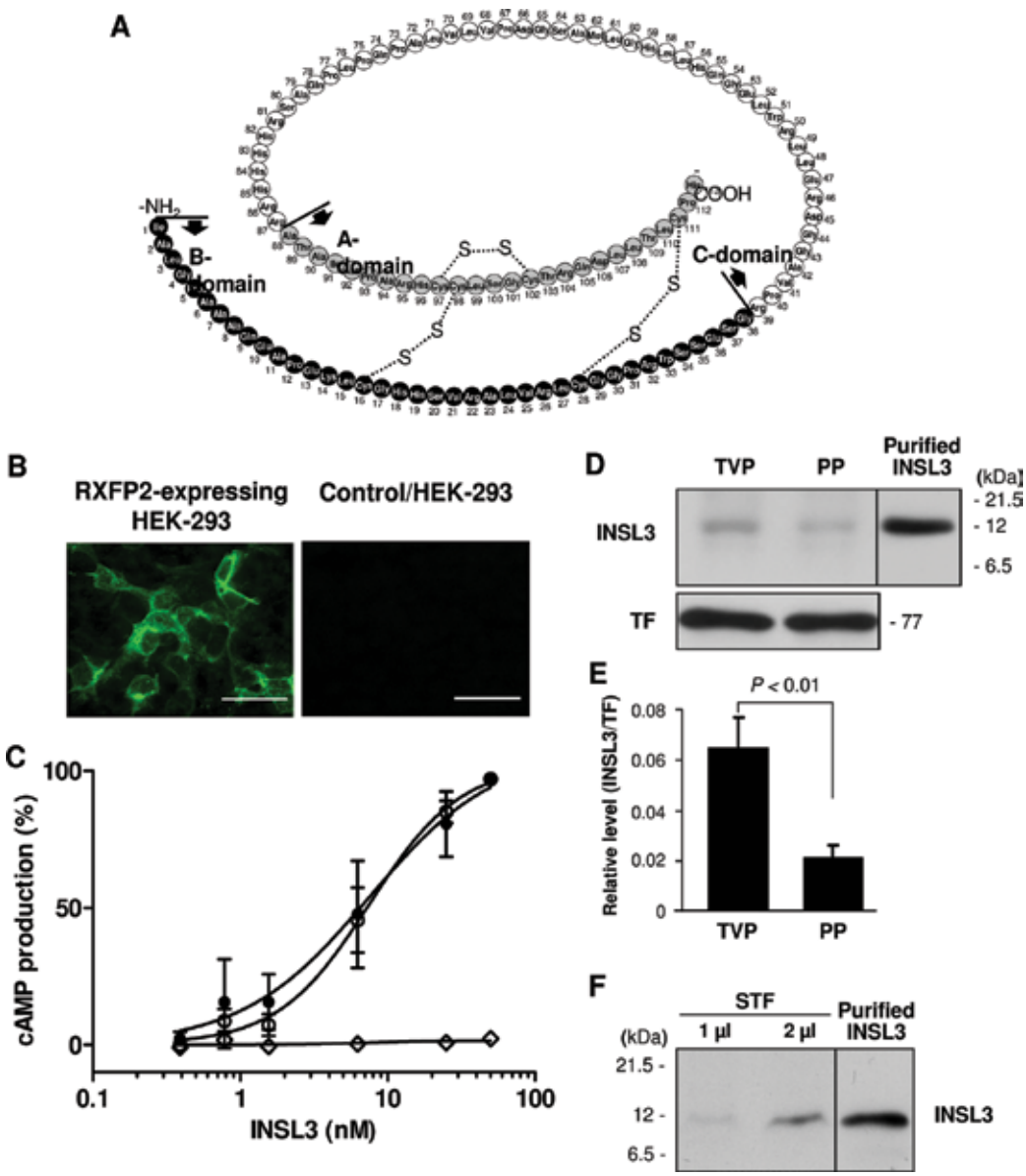

Figure 5. Structure, biological activity, and secretion of native goat INSL3. (A) Primary structure of goat INSL3 determined by MS/MS analysis. (B) RXFP2-expressing or control HEK-293 cells transfected with the expression construct or empty vector for detecting the biological activity of INSL3. (C) Stimulation of cAMP production by INSL3 in RXFP2-expressing HEK-293 cells. Purified goat INSL3 (●) stimulated dose-dependent cAMP production with EC_{50} values comparable with those of the synthetic A–B heterodimeric human INSL3 (○), indicating the retention of full bioactivity. In contrast, purified INSL3 did not stimulate cAMP production in the control/HEK-293 cells (◇). (D) A representative western blot analysis showing secretion of INSL3 into testicular venous plasma (TVP) and peripheral plasma (PP). (E) The relative level of each message normalized to that of TF (transferrin). INSL3 level was three-fold higher in TVP than that in PP. (F) A representative western blot analysis showing secretion of INSL3 into seminiferous tubular fluid (STF). Data are derived from Siqin et al. [25].

native goat INSL3 and its functional characterization would be a major step toward developing a specific immunoassay system for measuring INSL3 in blood and body fluids, whereby the yet unidentified endocrine, paracrine, and/or autocrine aspects of INSL3 can be explored, thereby giving insight into the potential role of INSL3 in goat testicular function.

2.3.2. Biological activity and secretion of INSL3

INSL3 mediates its effect by stimulating cAMP production through its specific cell-surface receptor, RXFP2 [5]. A reliable bioassay system based on the cAMP production in HEK-293 cells transfected with either mouse or human RXFP2 has been employed to examine the biological activity of INSL3. Treatment of RXFP2-expressing HEK-293 cells with the goat INSL3 resulted in a dose-dependent increase in intracellular cAMP production with an EC_{50} values (approximately 7 nM) comparable with those of the synthetic A–B heterodimeric human INSL3 peptide (**Figure 4**), indicating the retention of full bioactivity (**Figure 5B** and **C**).

Furthermore, western blot analysis of testicular venous and peripheral plasma and also of seminiferous tubular fluid demonstrated that the INSL3 secreted was present as the 12 kDa of molecular nature in blood and body fluid, revealing that most secreted INSL3 is not processed (**Figure 5D–F**) [25]. These results establish that goat INSL3 is secreted from testicular Leydig cells into the blood and body fluids within the lumen of the seminiferous tubules as a biologically active 12-kDa B–C–A single-chain peptide. Therefore, the native goat INSL3 differ from insulin, in which the prohormone undergoes proteolytic processing to form the active hormone, but is quite similar to insulin-like growth factors (IGFs) [26], as well as native porcine INSL3 [24], in that the proforms are not processed into two-chain peptides and exert full bioactivity.

2.3.3. INSL3 regulation by constitutive secretory pathway

It is important to note what secretory pathways are responsible for regulating INSL3 production and why INSL3 is secreted as a single-chain peptide. It is now accepted that endocrine cells producing peptide hormone possess regulated or constitutive secretory pathways, and that most secretory proteins that are synthesized as prohormones on the RER are transported to the cis-Golgi network and then through the trans-Golgi network (TGN) [27]. In the regulated secretory pathway, prohormones are sorted into secretory granules for processing and storage before their release (**Figure 6**). In contrast, the constitutive secretory pathway is thought to be the default pathway by which prohormones exit the TGN and are rapidly released without storage [27, 28]. Moreover, the major proteolytic enzymes mediating protein processing are prohormone convertase 1/3 (PC1/3) in the secretory granules in the regulated pathway and furin in the TGN in the constitutive pathway [27, 29]. For example, relaxin, which belongs to the same family as INSL3, is stored in secretory granules [30–32], and they undergo processing by PC1/3 [33].

Immunoelectron microscopy demonstrated that INSL3 localizes to the TGN within the goat Leydig cells, where secretory storage granules were undetectable, suggesting that goat INSL3

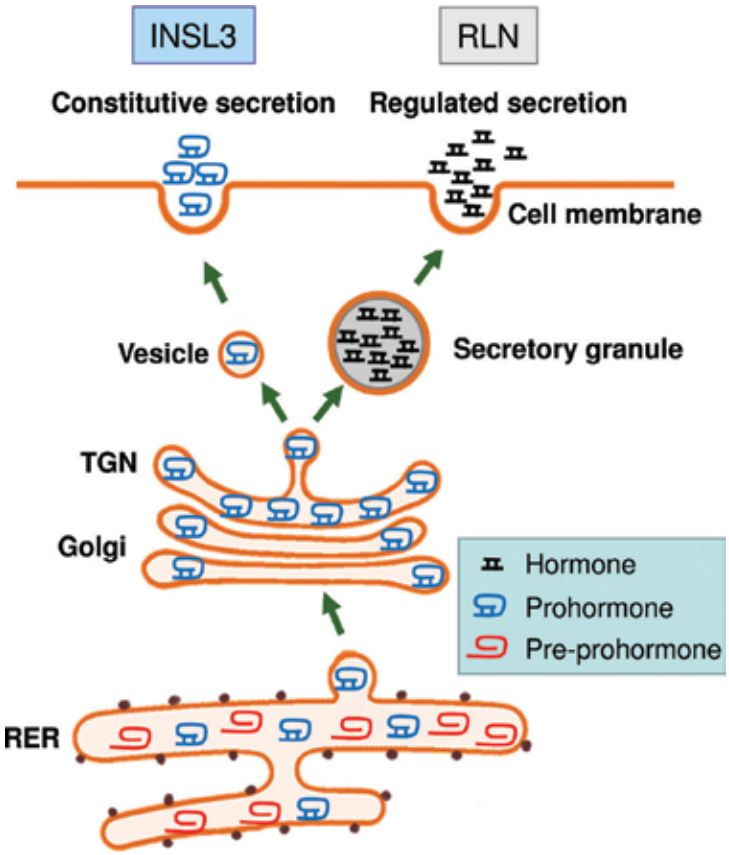

Figure 6. Regulation of INSL3 by constitutive secretory pathway. INSL3 and relaxin (RLN) are synthesized as preprohormone in rough endoplasmic reticulum (RER), converted to prohormone, and later transported to trans-Golgi Network (TGN). Pro-INSL3 is rapidly released by constitutive secretion without storage. In contrast, pro-RLN is packed into secretory granules, where pro-RLN is converted to RLN and condensation occurs before release. Based on our data [25, 30–32].

is not produced by the regulated secretory pathway but by the constitutive secretory pathway in Leydig cells (**Figure 6**) [25]. This is consistent with the findings that *INSL3* mRNA is constitutively expressed in rodent Leydig cells [13, 14, 20]. If this is the case, furin, rather than PC1/3, would mediate processing of INSL3 in goat testes. However, goat INSL3 did not actually have an RXK/RR sequence for cleavage catalyzed by furin [29]. Therefore, it is reasonable to conclude that goat testicular INSL3 is regulated by the constitutive secretory pathway and that the A–B heterodimeric INSL3 is not produced due to a lack of the RXK/RR motif at the furin cleavage site required for processing pro-INSL3.

2.4. INSL3-receptor RXFP2 network functions in testicular germ cells

2.4.1. An RXFP2-specific antibody that is suitable for detecting RXFP2

To investigate what effects INSL3 actually exerts in testis, the identification of the possible site(s) of action of INSL3 within the testis is necessary. Identifying the cell type(s) that

expresses the receptor RXFP2 and specific INSL3 binding in target cells would be a major step toward understanding the as-yet-unknown function of INSL3 in goats. Previous study determined the partial cDNA sequence of goat RXFP2 and demonstrated the high level of expression of this gene in mature testes, suggesting that INSL3 is involved in testicular function [34]. However, the specific cell type(s) that expresses RXFP2 in the testis have remained unidentified because of the absence of reliable anti-RXFP2 antibodies.

Recently, we successfully identified a highly specific anti-RXFP2 antibody that was suitable for detecting RXFP2 at protein level by analyzing the characteristics of commercially available RXFP2 antibodies that were directed against different parts of human RXFP2 [9, 35]. Identified RXFP2-specific antibody (GTX108235; GeneTex), which was directed against the human RXFP2 endodomain (C-terminal intracellular domain), recognized the cell-surface antigen by double immunofluorescence in HEK-293 cells transiently transfected with a FLAG-tagged mouse *Rxfp2* cDNA construct (HEK-293 expressing FLAG-RXFP2): RXFP2 signal overlapped with FLAG-RXFP2, which was recognized by the FLAG antibody, when the fluorescence images were merged (**Figure 7**). The characterization of the antibody has been described elsewhere [9, 16]. Notably, the antibody can react with not only functional 85-kDa receptor form that enables INSL3 to bind, but also smaller sized degradation forms, precursor forms, or splice variants of RXFP2, as verified by western blotting and far-western blotting of cell lysates from HEK-293 expressing FLAG-RXFP2 [9].

2.4.2. Germ cell types expressing RXFP2 mRNA and protein in the mature testis

Cell types expressing *RXFP2* mRNA and protein in the goat testis were identified by RT-PCR of cells captured using laser capture microdissection (LCM) and immunohistochemistry using the characterized RXFP2-specific antibody [16]. *RXFP2* transcripts were mainly expressed in spermatocytes and round spermatids, and in the Leydig cells (**Figure 8A**), whereas RXFP2 protein was detected specifically in the same cell types in which *RXFP2* mRNA was expressed

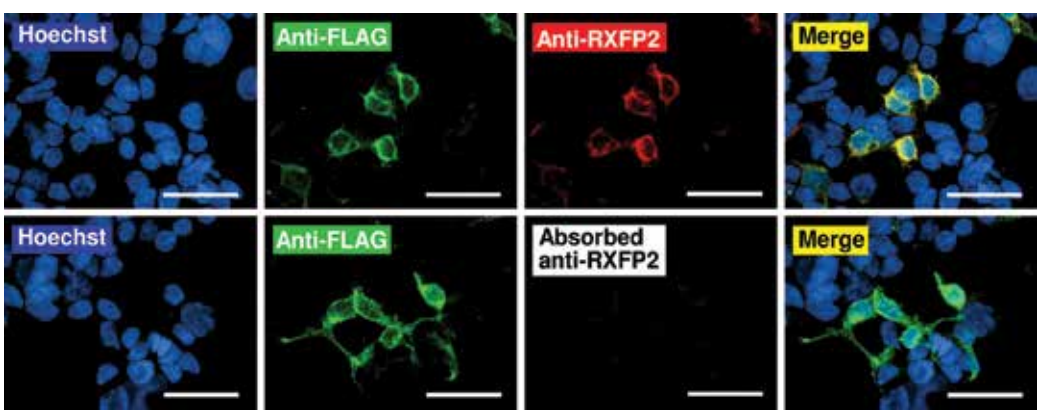

Figure 7. Verification of RXFP2 antibody specificity by double immunofluorescence labeling in HEK-293 cells transiently transfected with a FLAG-tagged mouse *Rxfp2* cDNA construct (HEK-293 cells expressing FLAG-RXFP2). RXFP2 signal overlapped with FLAG-RXFP2, which was recognized by FLAG antibody, when the fluorescence images were merged. In contrast, RXFP2 signal was abolished by preabsorbing the antibody with recombinant porcine RXFP2. Bars = 50 μm. Data are derived from Sagata et al. [9].

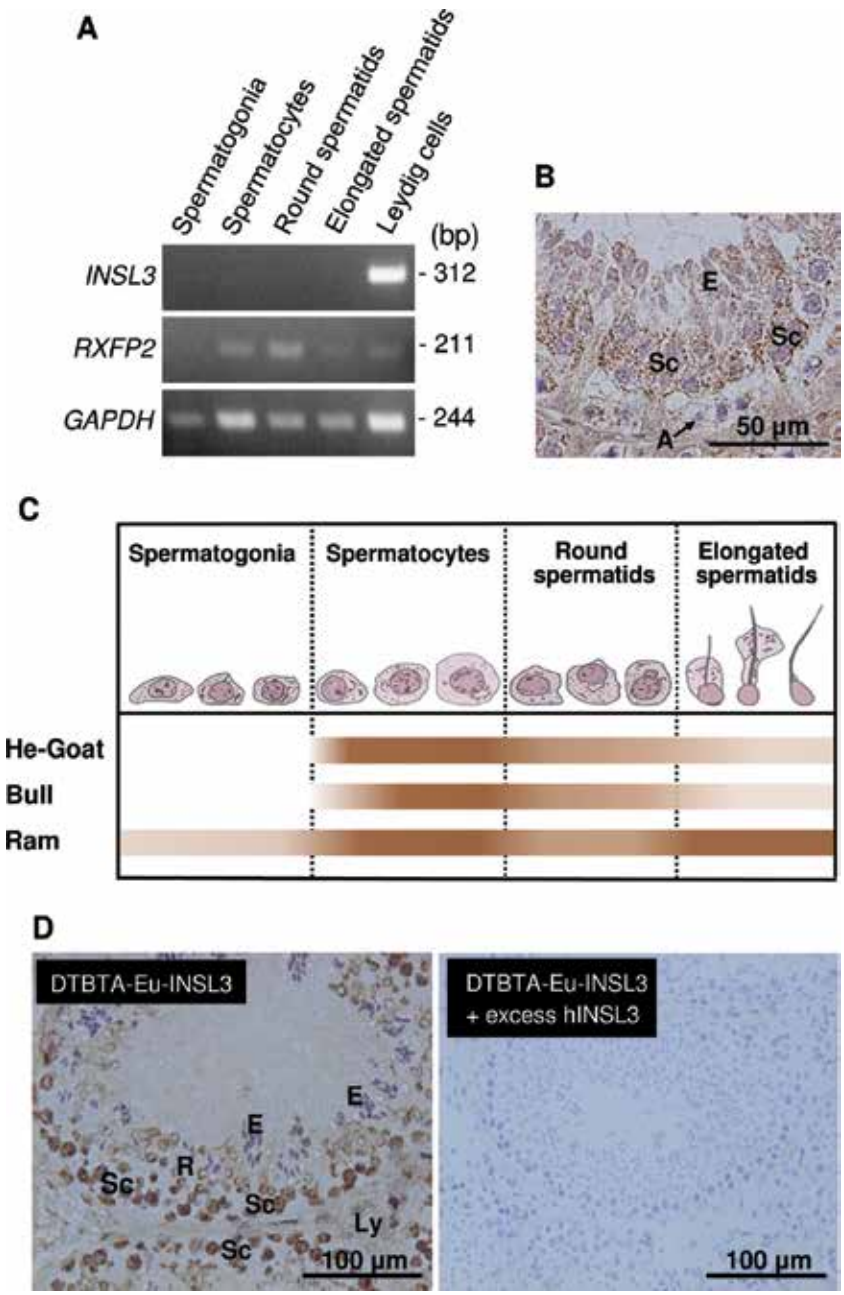

Figure 8. RXFP2 expression and INSL3 binding in germ cell in adult goat testis. (A) Specific cell types expressing *RXFP2* mRNA. Cells were collected by laser capture microdissection (LCM) microscopy and mRNA was detected by RT-PCR. *INSL3* transcripts were present only in Leydig cells, whereas *RXFP2* transcripts were highly expressed in Leydig cells, spermatocytes, and round spermatids and weakly expressed in elongated spermatids but not in spermatogonia. (B) A representative immunostaining of RXFP2 in the testis. (C) Comparison of RXFP2 protein expression during spermatogenic cycle among domestic ruminants. The brown colored bars indicate RXFP2 expression, with a darker shade reflecting a higher level of expression. (D) *In situ* INSL3 binding in germ cells. INSL3 binding was detected in germ cells but not in Leydig cells in sections incubated with DTBTA-Eu-INSL3. The binding was inhibited by excess hINSL3. A, type A spermatogonia; Sc, spermatocytes; R, round spermatids; E, elongated spermatids; Ly, Leydig cells. Data were derived from Pitia et al. [16, 36].

(**Figure 8B** and **C**). Especially, RXFP2 expression in meiotic and postmeiotic germ cells in goats are fundamentally consistent with our studies in boars [9, 35], bulls, and rams [36], as well as other reports in humans [37] and rats [8].

2.4.3. INSL3 ligand binding to germ cells

An *in situ* ligand assay with DTBTA-europium (Eu)-INSL3 was useful for determining whether INSL3 can bind RXFP2 expressed in Leydig and germ cells, since the binding of ligand to specific receptors on target cells initiates downstream physiological effects. DTBTA-Eu, which is a recently developed luminescent lanthanide chelate label, has several advantages such as not interfering with the biological activity of the labeled proteins [15]. In goat testes, INSL3 mainly binds to spermatocytes, with weak binding of round and elongated spermatids, in a hormone-specific and saturable manner (**Figure 8D**) [16]. Unexpectedly, no binding of INSL3 to Leydig cells was detected, despite their expression of *RXFP2* mRNA and protein, indicating that a functional RXFP2 receptor is expressed only in germ cells.

As shown in **Figure 5D–F**, goat INSL3 secreted from testicular Leydig cells was released not only into the blood circulation but also into seminiferous tubules [25]. This finding suggests that INSL3 enters the seminiferous compartment across the blood-testis barrier (BTB), which is created by inter-Sertoli tight junctions and adherens junctions, by mechanisms which are still unclear. Similar findings were also found in boar and rat INSL3 [9, 14]. These results taken together reveal that INSL3 secreted from the Leydig cells is transported into the seminiferous compartments and is capable of encountering germ cells, where its receptor RXFP2 is expressed, thus enabling INSL3 to bind (**Figure 9**). This suggests that INSL3 could act as a paracrine factor in seminiferous germ cells in the goat testis. Unfortunately, it remains unknown what functions INSL3 actually exerts on germ cells in the goat testis. One possibility is that INSL3 plays a role in the maintenance of spermatogenesis by regulating cellular processes such as apoptosis through the receptor RXFP2 on seminiferous germ cells in a paracrine manner. In fact, there is compelling evidence that INSL3 contributes to the maintenance of spermatogenesis by acting as a germ cell survival/antiapoptotic factor in testes of rats [8] and boars [9], and as a germ cell proliferation factor in zebrafish [10].

In contrast, the reason why INSL3 did not bind to Leydig cells is unclear, despite their expression of *RXFP2* mRNA and protein. However, there is the possibility that RXFP2 receptors in Leydig cells might have been saturated with endogenous INSL3 *in vivo*, leading to their long-term desensitization/internalization [38]. Another possibility is the expression of RXFP2 splice variants in Leydig cells, as shown in rodent testes [37]. In fact, RXFP2 variants that lack part of the ectodomain (N-terminal extracellular domain) components that are essential for ligand-receptor interaction often yield nonfunctional products [39, 40].

2.5. The potential of INSL3 hormone-receptor system as a novel parameter for predicting fertility in breeding sires

2.5.1. Expression and functionality of RXFP2 in the spermatozoa

INSL3 possibly plays a role in sperm function as suggested by the detection of its receptor *RXFP2* mRNA or protein in spermatozoa of rodents [41] and boars [42]. However, there

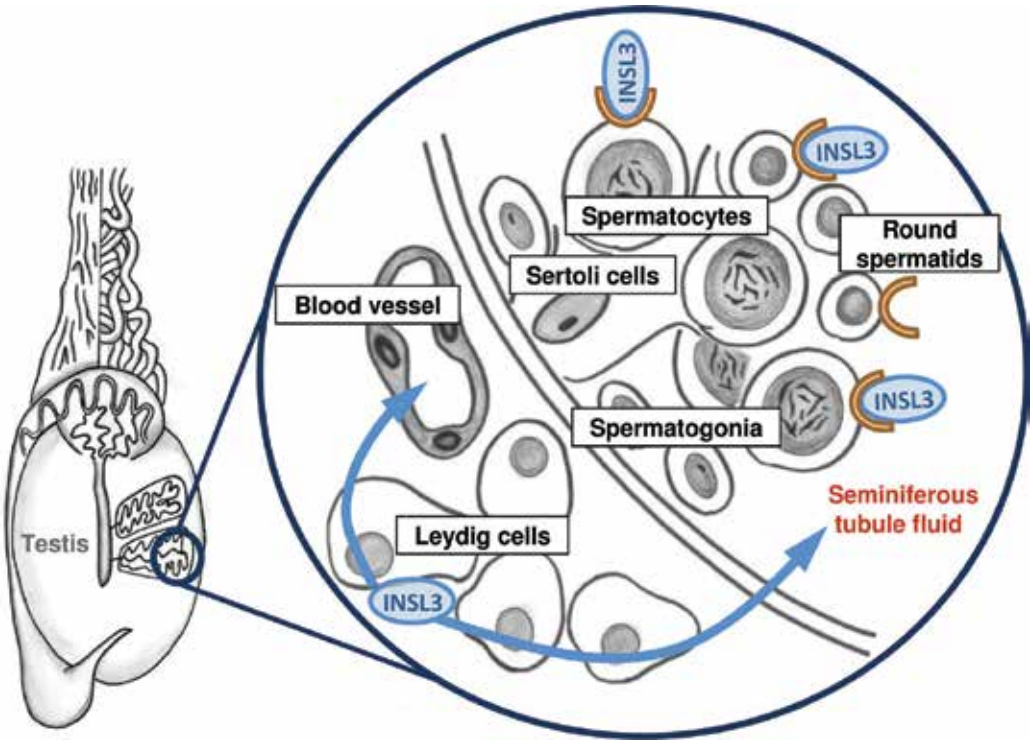

Figure 9. INSL3 secreted from Leydig cells is released not only into the blood circulation, but also into the interstitial and seminiferous compartments, where receptor RXFP2 is expressed mainly in the germ cells, suggesting its involvement as a paracrine factor. Based on our data [16, 25].

was no evidence whether RXFP2 is expressed in spermatozoa of domestic ruminants, including goats. With the help of immunofluorescence using RXFP2-specific antibody, we found that RXFP2 was expressed in the equatorial segment and midpiece of goat spermatozoa (**Figure 10A**). Similar findings are also found in bull and ram spermatozoa [36].

Furthermore, we investigated the functionality of RXFP2 in these spermatozoa using *in situ* ligand binding assay with DTBTA-Eu-INSL3 and clearly showed the presence of functional RXFP2 enabling INSL3 to bind with the equatorial segment of he-goat (**Figure 10B**), bull, and ram spermatozoa, suggesting that a functional INSL3 hormone-receptor system operates in the equatorial segment of ruminant spermatozoa [36]. It has been reported to date that *RXFP2* mRNA is detected in the spermatozoa of rats [41] and boars [42] and its protein is distributed throughout the entire spermatozoon in boars [43]. However, the reason for discrepancy in receptor distribution is unclear, but it might be due to differences in animal species and antibodies used. It is important to note where INSL3 acting on spermatozoa comes from. INSL3 has been reported to be produced in theca cells in the ovary of some species such as rats [8] and cows [44]. In addition, high levels of INSL3 is detected in follicular fluid of rhesus macaques [45]. Therefore, INSL3 in follicular fluid entered into oviduct at the time of ovulation appears to operate in spermatozoa.

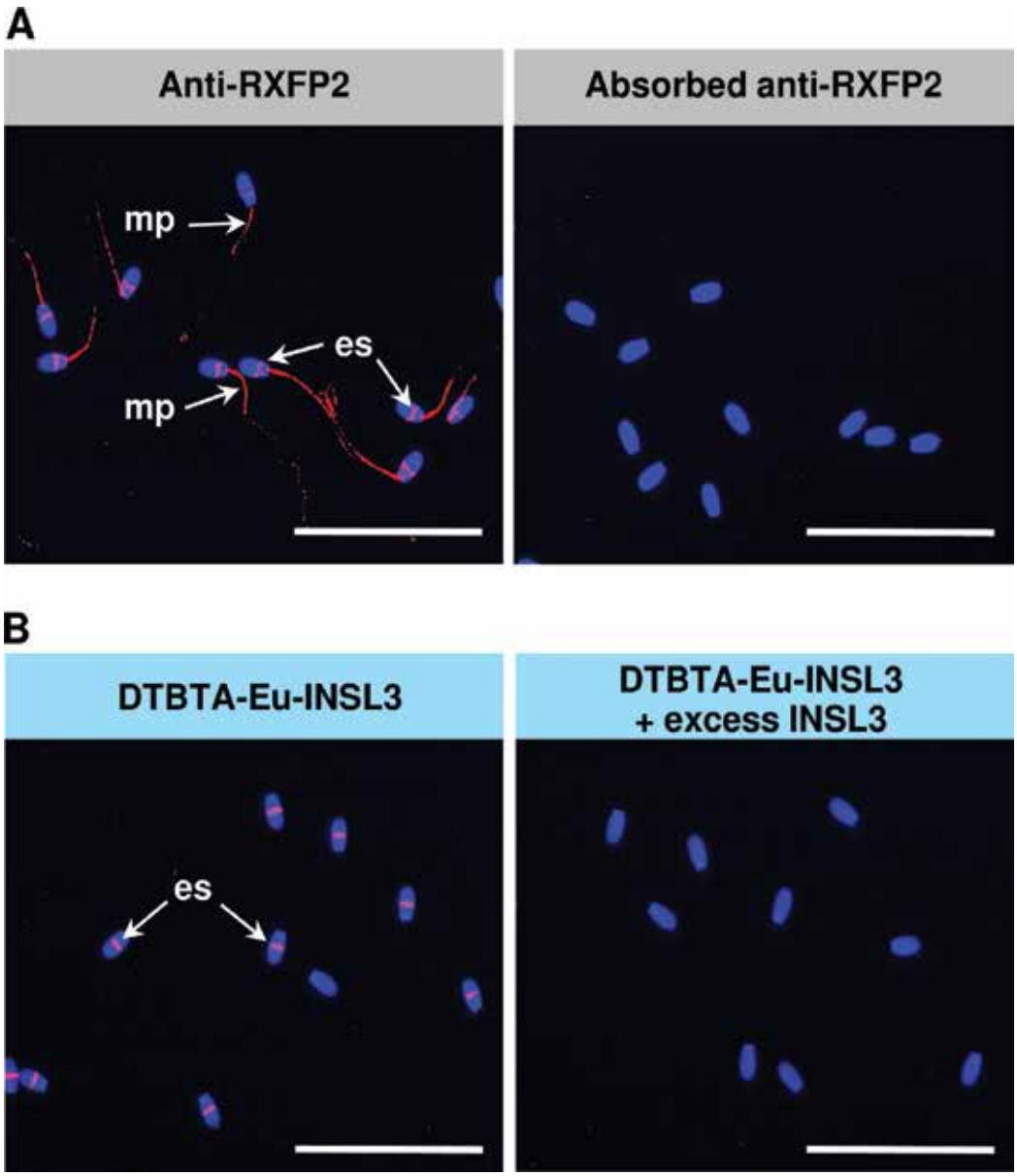

Figure 10. Expression and functionality of RXFP2 in the goat spermatozoa. (A) Immunolocalization. RXFP2 signals were observed in the equatorial segment and midpiece of the spermatozoa, and were blocked using the preabsorbed antibody. (B) *In situ* INSL3 binding. INSL3 binding was exclusively detected in the equatorial segment of goat spermatozoa incubated with DTBTA-Eu-INSL3. The binding was inhibited in the presence of excess hINSL3. es, equatorial segment; mp, midpiece. Bars = 50 μm. Data were derived from Pitia et al. [36].

Although the physiological significance of RXFP2 in the equatorial segment of ruminant spermatozoa, including goats, is still unknown, it might be related to sperm fusion with the oocyte membrane during fertilization. A recent study on knockout mice has demonstrated

that gamete fusion is mediated by β-catenin through formation of β-catenin/E-cadherin complex, which facilitates adhesion on both sperm and oocyte plasma membranes [46]. Both β-catenin and E-cadherin are expressed on the acrosomal and/or midpiece regions of the spermatozoa in bulls [47], humans [48], and mice [46]. As INSL3 appears to activate the Wnt/β-catenin canonical pathway through RXFP2 in gubernaculum cells [49], it seems reasonable to assume that INSL3 may contribute to gamete fusion by upregulating β-catenin required for formation of β-catenin/E-cadherin complex via RXFP2 in the equatorial segment of ruminant spermatozoa. The lack of INSL3 binding to the midpiece could be attributed to the expression of RXFP2 splice variants therein, although there is currently no evidence for such variants in the spermatozoa of any species. If they are indeed present, RXFP2 splice variants may affect receptor functionality and prevent INSL3 binding in spermatozoa.

2.5.2. Potential to diagnose subfertility in sires

Reproductive efficiency in domestic ruminants is a major factor that affects profitability in meat and dairy industries. In sires, normal sperm production and good semen quality are essential in maximizing reproductive performance. Artificial insemination (AI) with frozen semen has been employed in modern breeding programs of dairy and meat livestock to improve economically important traits. Especially, in cattle, it is prominent. However, even when semen quality parameters such as sperm viability and motility are good, some prized sires are still adversely affected by low fertility (subfertility). Thus, it is important to explore novel molecular markers for an accurate assessment of male fertility from the perspective of sperm production and semen quality.

Although serum INSL3 levels are positively correlated with sperm production [50], sperm number [50], and sperm morphology [51] in humans, we examined the potential to diagnose subfertility from the spermatozoa [36]. Since samples suitable for this analysis were available in bulls, we used bull spermatozoa as an alternative to goat spermatozoa. *In situ* ligand binding assay revealed that the functional receptor RXFP2, enabling INSL3 to bind, was expressed only in the equatorial segment of both fertile and subfertile spermatozoa (**Figure 11A**). However, when sperm population expressing the functional RXFP2 receptor was evaluated, the percentage of INSL3-labeling spermatozoa was significantly reduced ($P < 0.05$) in the semen of subfertile bulls compared to that of fertile bulls (**Figure 11B**). This suggests the possibility to diagnose subfertility in bulls based on sperm population expressing the functional RXFP2 receptor. To date, many proteins such as seminal plasma protein, α-L-fucosidase, cathepsin, clusterin, fertility-associated antigen, or osteopontin might be useful fertility markers in bulls [52]. Additionally, the immunoassayable levels of hormones, such as follicular stimulating hormone, testosterone, and RLN, have also been suggested as useful indices for predicting the fertility potential of sires [53–56]. Thus, the present evaluation, based on the properties of the INSL3 hormone-receptor system in the spermatozoa of ruminant sires, might represent an effective means by which fertilizing potential can be assessed from the standpoint of sperm quality, respectively.

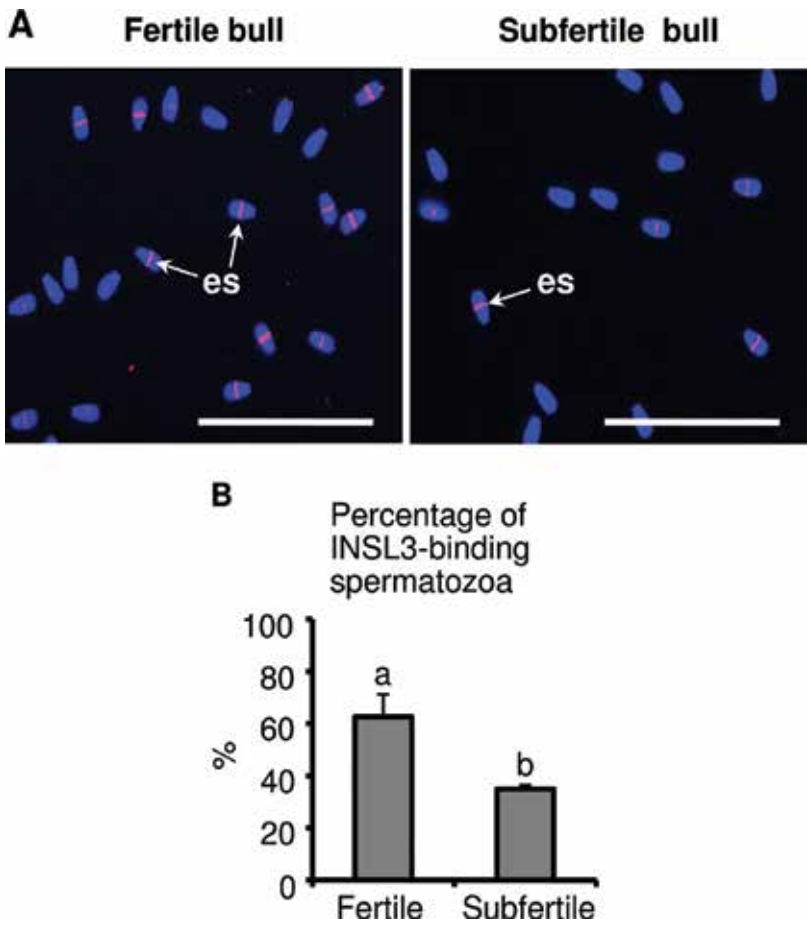

Figure 11. Evaluation of sperm population expressing the functional RXFP2 receptor enabling INSL3 to bind in the semen of fertile and subfertile bulls. (A) Binding of INSL3 in bull spermatozoa *in situ*. INSL3 binding signals were visualized only in the equatorial segment of both fertile and subfertile bull spermatozoa. es, equatorial segment. Bars = 50 μm. (B) Morphometric analysis. Percentages of INSL3-binding spermatozoa were significantly reduced in the semen of subfertile bulls compared to that of fertile bulls. Values are expressed as mean ± SE, and values with different letters are significantly different ($P < 0.05$). Data were derived from Pitia et al. [36].

3. Conclusions

While peptide hormone INSL3 is essential for fetal testis, and possibly acts as an important player in testicular and sperm functions in adult males, there has been very little progress in understanding the functions of INSL3 in male ruminants, including goats. In this review, we have undertaken an understanding of both the structure and function of INSL3 in male goats to fill a gap in our knowledge. From a structural point of view, we provided evidence that goat INSL3 is constitutively secreted from Leydig cells as an unprocessed B–C–A form

retaining the C-domain with full biological activity. The reason why the INSL3 exists as a B–C–A form is unclear, but the C-domain does not appear to interfere with receptor binding and activation. Additionally, clarification of native goat INSL3 will facilitate development of a specific immunoassay system for monitoring INSL3 in blood and body fluids. In contrast, from a functional point of view, we provided the evidence for a functional receptor that binds INSL3 in testicular germ cells and in spermatozoa, implying that the intra- and extratesticular INSL3 hormone-receptor system operate in male goats. We also found the potential of this system as a novel parameter for predicting fertility in breeding sires. However, it remains unknown what functions INSL3 actually exerts on testicular germ cells and on spermatozoa in this species. Finally, the findings outlined here will help in the discovery of new target tissues/organs and receptor-expressing cells, not only in male goats but also in female goats, thereby giving insight into the potential role of INSL3 in those organs.

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Chapter 7

Estrus Synchronization and Artificial Insemination in Goats

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Abstract

Goats are small ruminants found worldwide. They provide humans with meat, milk and skin. In many rural communities, goats serve as a store of economic value and are used in cultural celebration. The world population in rapidly growing and is predicted to reach 9.6 billion by 2050. Human population explosion will exert immense pressure on the availability of food resources. Goats provide an excellent source of food to feed the world growing population. In order to increase goat population, advanced reproductive biotechnologies must be employed. These methods include and it not limited to estrus synchronization artificial insemination. Estrus synchronization is achieved by manipulation of the estrous cycle using exogenous hormones such as progestagens, gonadotrophins, and prostaglandins. Artificial insemination can be described as all the processes involved in semen collection from a male, evaluation, processing, and eventual deposition in the vaginal of a suitable female to cause conception. Adequate knowledge about male and female reproductive anatomy and physiology is critical to the application and success of reproductive biotechnology in goat reproduction.

Keywords: estrus synchronization, artificial insemination, goats, progestagens, prostaglandin, semen

1. Introduction

Food is one of the basic necessities of life besides clothing and shelter. Animals provide a rich source of nutrients for humans. With the increasing world population and limited natural resources, many strategies to improve the reproductive capacity of domestic livestock is progressively being explored to cater for the needs of humans. Goats are hardy small ruminants that have the potential to provide meat, milk and hides [1]. Food products from goats are

a delicacy in many parts of the world. However, goats have been somewhat neglected by the research community compared with other livestock species such as cattle, poultry and sheep. Goats are hardy, have high tolerance to heat stress and can survive harsh conditions. Also, goats contribute on the preservation of the ecosystems and can be used as an ecological tool for controlling the noxious weeds, reducing the incidences of wildfire, improving the rangelands and wild life habitat [2]. In many parts of the world, goats serve as a store of wealth and are used in many cultural activities. Interestingly, the male goat is seen as a symbol of fertility. Indeed, the importance of goats in the teeming efforts to ensure regional protein sufficiency and world food security cannot be overemphasized.

Efforts to multiply goats by the application of reproductive biotechnology is on the increase especially in developed countries. In fact, goat milk is a huge industry in North America and some parts of Asia. In Africa, goat meat is considered premium meat and is associated with higher prices. Goats are important in development because of their ability to convert forages and crops and household residues into meat, fiber, skins and milk [3]. Of the different biotechnology techniques viz. multiple ovulation, in vitro fertilization, embryo transfer, etc., estrus synchronization and artificial insemination (AI) are the most powerful biotechnology tools that have hasted genetic progress and enhanced fertility in goats and farm animals [4]. Adequate understanding of these tools cannot be overemphasized and must be carefully implemented in order to ensure breeding success. Reproduction is critical to success of any livestock enterprise, including goat rearing. The objective of this mini review is to describe the use of estrus synchronization and artificial insemination techniques to enhance reproductive performance of goats.

2. Materials and methods

To achieve our stated objective, a narrative review was carried out in February 2016. The database searched was PubMed and Google search. Search terms were "estrus synchronization in goats" and "artificial insemination in goats." A total of 301 articles were retrieved from the search out of which 146 were duplicates. These studies were carried out using different breeds of goats treated with varying hormones and protocols. Results of estrus synchronization studies carried out by the author in tropical environments were also included in the final write-up.

3. Estrus synchronization in goats

Estrus synchronization enables concentrated breeding that ensures uniform kid crop and proper management of pregnant does. Exogenous hormones are used to modify the physiological chain of events involved in the sexual cycle, while the non-hormonal methods of OS involve the use of light control or exposure to a male. In the doe, the window of opportunity is generally greater during the luteal phase, which is of longer duration and more responsive to

manipulation [5, 6]. Estrus synchronization protocols utilize several different hormones in sequence to control CL function, stimulate follicular development and regulate ovulation.

3.1. Prostaglandins and their synthetic analogues

An easy-to-apply method of estrus synchronization in goats is by the use of prostaglandins to cause luteolysis so as to induce the subsequent follicular phase of the estrous cycle. In small ruminants, prostaglandin $F_{2\alpha}$ is the primary luteolytic agent [7]. Since consumers demand food produced by "clean, green and ethical" methods [8], prostaglandins are a good alternative to progestagens. This is because prostaglandins are rapidly metabolized in the lungs and therefore, do not accumulate in tissues [9]. Prostaglandins are mainly administered intramuscularly and subcutaneously, although the intravulvo-submucosa route has been investigated with varying success. Several synthetic analogues have been used to induce rapid regression of the corpus luteum. Although natural $PGF_{2\alpha}$, causes normal luteolysis through gradual degenerative changes, synthetic analogues of $PGF_{2\alpha}$ usually have a more rapid and dramatic effect on progesterone synthesis in the lutein cells [10]. Dinoprost thromethamine marketed as Lutalyse® and Carboprost® are frequently used natural prostaglandins, while cloprostenol sodium, marketed as Fenprostenol®, Estrumate® and estroPlan®, is a synthetic prostaglandin (**Figure 1**) [11, 12]. Factors reported to affect estrus response and subsequent fertility following administration of prostaglandin or its analogues include the dose level of the prostaglandin [13], the interval between administration of the prostaglandin [14], the responsiveness of the corpus luteum to the prostaglandin/stage of the oestrus cycle [15], season and the inclusion of gonadotrophins as co-treatment [16]. Several gonadotrophins such as follicle-stimulating hormone (FSH), pregnant mare serum gonadotrophin (PMSG) and gonadotrophin-releasing hormone (GnRH) have been included in the prostaglandin protocols, resulting in improved estrus response rates. Prostaglandins should be administered from day 3 of the oestrus cycle, when the corpus luteum of the goat is responsive to $PGF_{2\alpha}$ [17].

Prostaglandins have the major advantage of being administered by intramuscular injection besides the reduction in hormonal residues, since it is rapidly and almost completely metabolized in the lungs [18]. Following prostaglandin administration, compromised follicular function has been reported leading to variability in the timing of ovulation [19]. It is essential that two injections of prostaglandin $F_{2\alpha}$ is administered 9–11 days apart. By so doing, almost all the animals would be in the mid luteal phase of the oestrus cycle and would better respond to the second treatment [20]. Double treatment with cloprostenol sodium administered i.m., 11 days apart, resulted in higher oestrus response (92.8% versus 75%) than single treatment in Red Sokoto does [21].

3.2. Progesterone and its synthetic analogues

Another method of estrus synchronization is by the use of natural progesterone impregnated in sponges, implants or silicon elastomers, or the use of its synthetic analogues such as norgestomet, fluorogestone acetate (FGA), methyacetoxy progesterone (MAP) and medroxyprogesterone acetate (MPA) [22]. The progesterone or progestagen treatment is popularly delivered though an intravaginal sponge, intramuscular or subcutaneous routes. Natural progesterone is

Figure 1. Some hormones used for estrus synchronization in goats. Hormones are mostly administered via the intramuscular, intravaginal, oral or intradermal route (picture by B.O. Omontese).

mainly marketed as Sil-Oestrus® implant and Eazi-Breed® controlled internal drug release devices™ (CIDR) (**Figure 1**). Synthetic analogues are marketed as Chronogest® (Intervet, Angers, France) and Veramix sponges® (Pharmacia & Upjohn, Orangevillle, Canada). Traditionally, intravaginal sponges are inserted over periods of 9–21 days and in most cases, eCG or $PGF_{2\alpha}$ is administered 2 days before at the end of pessaries removal. Factors that affect the success of an OS programme when progestagens are applied include species, breed, co-treatment, management, stage of the oestrus cycle, duration of treatment and mating system.

The use of long-term progestagen treatments has been shown to result in lowered fertility rates in goats [22]. On the other hand, decreased periods of progestagen treatment may minimize vaginal discharge and infection, and increase fertility. Currently, short-term intravaginal progestagen treatment is advocated. Following withdrawal, does usually show overt oestrus within 48 h. More recently, an alternative means of supplying continuous, exogenous progesterone has been the CIDR's, developed for sheep and goats in New Zealand. It is made from medical

silicone elastomer molded over a nylon core and impregnated with natural progesterone (330 mg). CIDRs are preferable than sponges because they are easy to use, do not cause as much discomfort as sponges and do not adhere to the vaginal wall during use. The addition of gonadotrophins to progestagen protocols ensure a tighter synchrony and/or induces a superovulatory response in treated does [8]. The use of gonadotrophins increases the cost of oestrus synchronization and is reported to reduce fertility of does in the long-term. Besides, repeated administration of eCG is reported to produce antibodies against eCG (anti-eCG), thereby causing reduced ovarian stimulation after subsequent treatments [23].

4. Artificial insemination in goats

Artificial insemination (AI) is defined as the process by which sperm are collected from the male, processed, stored and artificially introduced into the reproductive tract of a female for the purpose of conception. It is essentially the most important techniques for genetic improvement of farm animals. Although AI is most widely used for breeding dairy cattle, it is an indispensable tool for genetic improvement in small ruminants and poultry. AI has an interesting history; from the Arabian chieftain who introduced a wand of cotton into a mare's reproductive tract to collect semen in 1322 A.D., to Anthony van Leeuwenhook who first observed human spermatozoa under magnification, to Spallanzani who is described as the inventor of AI for successfully conducting AI in dogs and the Russian scientist Ivanoff who pioneered AI research in birds, horses, cattle and sheep, and was the first to successfully artificially inseminate cattle. Artificial insemination is and continues to be an essential reproductive technique for genetic purposes including creation and diffusion of genetic progress and conservation of genetic resources.

4.1. Advantages and disadvantages of AI

AI has several advantages of which the greatest is the ability to maximize superior sires for genetic improvement. AI prevents spread or exposure of sires to infectious genital diseases. In addition, bull evaluation that accompanies AI enables early detection of infertile bulls, eliminated handling of stubborn bulls and allows use of bulls unable to mount due to foot injuries. Importantly AI also helps ensure that accurate breeding records can be kept. As with many scientific techniques, the disadvantages of AI include increased cost associated with labor and facilities. In addition, AI only allows utilization of a few sires, which reduces genetic base. Also, there is potential for rapid spread of undesirable traits, if bucks from which semen is sourced are not carefully evaluated, hence, if the buck had a genetic defect this will be widely spread in the population. Therefore optimum care and critical evaluation of semen from bucks to be used for artificial insemination is of paramount importance.

4.2. Collection, extension and storage of semen

For AI to be successful, quality semen must be used. The quality of semen is determined by proper collection, extension and storage. Several methods of obtaining semen have been

developed but the most popular method in goats is the use of electro ejaculator and artificial vagina. The later method, which gives the best quality, requires that the stud properly stimulated and then allowed to mount a teaser doe and ejaculates when the penis is directed into the artificial vagina. When an electroejaculator is used, rhythmic stimulation of the ampullae, accessory sex organs and the sacral nerve plexus cause erection and ejaculation. Semen should be evaluated for mass motility, individual progressive motility, volume, pH, ejaculate concentration and morphology. It is important that a strict sense of hygiene is maintained during the process of semen collection, processing and storage. Diluting/extending semen increases the volume and helps to increase the number of females serviced from one ejaculation as a normal ejaculate from a buck varies from 2 to 6.5 × 10 sperm/ml. Examples of commercially available semen extenders are sodium citrate diluent, Tris diluent, Cornell Union Extender, egg yolk-phosphate [24]. Other non-conventional extenders include homogenized milk-fructose, homogenized whole milk-phosphate, Tris-coconut milk and coconut milk citrate [25]. Cryoprotectant glycerol, and antibiotics such as penicillin and streptomycin are added to semen extenders to inhibit bacterial growth [26]. Semen is stored for short term or long term depending on when it should be used. It can be refrigerated (+4°C) for short term or in liquid nitrogen for long term (−96°C). Semen stored in liquid nitrogen can be viable for decades with limited deterioration in fertility.

4.3. Insemination technique

There are three methods of inseminating goats; intracervical, intrauterine (difficult), or laparoscopic insemination. For fertility to be optimum, semen must be deposited in the appropriate place in the reproductive tract of the female and at the right time (when a female is in estrus). In does, semen may be deposited in the vagina but the best location to optimize fertility is when semen is deposited in the inner cervix. Insemination in does requires the use of an insemination gun, pipette and vaginal speculum. AI guns are also referred to as pistolettes. There are three sizes of AI guns; 0.25 cc, 0.5 cc and Universal. A loaded AI gun consists of a plunger which forces a plug through the straw to expel semen, a barrel which houses the semen straw, disposable plastic sleeve (sock or sheath)—to keep the semen straw in place, semen straw and an O-ring-to maintain the inseminating sheath in place. Does must be restrained; vulva should be cleaned before inserting the inseminating gun. Semen should be slowly deposited near the uterine end of the cervix or just inside the uterus. The ease of inseminating goats varies, as it is simpler with older, multiparous does with larger and less convoluted cervix compared with younger does with smaller and highly convoluted cervix.

5. Conclusion

Goats continue to provide of nutritional, economic and social benefits. Reproductive biotechnologies should be adopted to improve the overall efficiency of goat production systems. Estrus synchronization and artificial insemination continue to be essential reproductive techniques for genetic creation, diffusion and conservation of genetic resources.

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Milk Production

Chapter 8

Proteomic Analysis of Goat Milk

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Abstract

The advancement of electrophoresis and chromatography, along with technological developments in mass spectrometry, has widened the potential application of proteomics to study milk from smaller ruminants. The aim of this chapter is to provide an in-depth overview of the development and progress of proteomics applications in goat milk. After examining various proteomic approaches that are currently applied to this field, we narrow our focus on proteomic investigations of mastitis in goat milk. A summary of protein modulation in goat milk during experimentally-induced endotoxin mastitis is discussed. Because the molecular function of proteins is disrupted during disease due to changes in post-translational modifications, we also review the phosphorylation of caseins, which are the predominant phosphoproteins in milk, and discuss the implications of casein modifications during mastitis. These results offer new insights into the changes of protein expression in goat milk during infection.

Keywords: goat milk, mastitis, proteomics, casein phosphorylation

1. Introduction

Milk is an important biological fluid and an essential nutrient for young mammals and humans during their lifetime. It provides macro- and micro-nutrients and is an important source of antimicrobial and immunoregulatory agents [1]. Typically, the U.S. dairy industry relies on cows as the main source of milk and other dairy products. However, cow milk has been implicated in the increasing rates of protein allergies in infants [2, 3], causing gastrointestinal disorders in adults [4], and contains insufficient concentrations of iron [5], thus an interest in finding alternatives to cow milk has emerged.

Goat milk is an excellent source of macro- and micro-nutrients, and proteins that are more easily digested, presumably due to the higher essential fatty acid contents [6]. It is also less allergenic than cow milk, as it was shown to help children at risk of food allergies [7]. While not very popular in the United States, in developing countries where cow milk is not readily available or affordable, goat milk accounts for more than 50% of milk production [8]. In Europe, goat milk is processed mostly for cheese manufacturing [9]. Considering the economic importance in developing countries and the inherent health benefits, goat milk might be a reliable alternative, if not a replacement, for cow milk [10].

Proteins are the key components of milk with many diverse cellular functions. For example, casein micelles provide essential amino acids that are vital for energy, tissue growth, and cellular function. In addition, some proteins can act as hormones, whereas others display antimicrobial properties. Proteomics is the large-scale study of the protein contents of cells and tissues [11]. One of the most promising outcomes of proteome analysis is the discovery of protein biomarkers, which are specific proteins or protein isoforms, whose expression levels change significantly during disease conditions [12]. The identification of these biomarkers in accessible body fluids such as milk could eventually enable farmers and veterinarians to monitor diseases and expand treatment options.

Mastitis, the focus of several prior veterinary proteomics studies, is defined as inflammation of the breast or udder tissues that is typically caused by invading bacteria. The first proteomic investigation of bovine milk mastitis was conducted by Baeker et al., where they used a comparative proteomics to identify expressed proteins in normal and mastitic bovine milk [13]. This was followed by several other research investigations to identify differentially expressed proteins in cow milk, either following experimentally induced infection or during naturally occurring mastitis [14–16]. Quantification of expressed proteins in clinically healthy cows and cows with experimentally-induced coliform mastitis was also reported using both liquid chromatography tandem-mass spectrometry (LC-MS/MS)-based label-free approach [17] and isobaric peptide tags for relative and absolute quantification (iTRAQ) [18]. As a result, biochemical mechanisms and inflammatory-related biomarkers, especially acute phase proteins (APPs), were identified [19–21].

Like other ruminant species that are managed for milk production, goats are also affected by mastitis; however, much less is known about the goat innate immune response to mastitis pathogens or about subsequent changes in goat milk protein expression over the course of a clinical infection. Although some of the apparent clinical signs of mastitis in goats, including udder swelling and redness, increased rectal temperature, and changes in the appearance of the milk are similar to those observed in dairy cattle, limited knowledge of the host response during mastitis in goats exists. Nonetheless, other hallmarks of clinical mastitis in dairy cattle include elevated milk somatic cell counts (SCC), reduced appetite, reduced milk production, increased heart rate, and changes in blood chemistry [22, 23].

Initial reports of proteomic evaluations of goat milk were limited to the analyses of casein fractions and the determination of the molecular weights of major whey proteins [8, 24]. Later attempts to identify differentially expressed proteins in goat milk employed gel-based assays followed by enzymatic digestion of isolated proteins and matrix-assisted laser desorption/ionization

mass spectrometry MALDI-MS [25, 26] or further refinements of gel-based assays followed by LC-MS/MS [27]. Until recently, no studies have focused on the proteomic analysis of goat milk protein modulation during clinical mastitis. The first study of goat milk protein modulation over the course of experimentally-induced mastitis using proteomics was recently been reported by our group [28]. Since the molecular function of proteins can be disrupted during the disease due to the changes in post-translational modifications (PTMs), we also evaluated the phosphorylation status of caseins [29].

This chapter will provide an overview of common proteomic approaches and the application of proteomics to the detection of caprine milk proteins. Likewise, a brief description of the advances in our current understanding of goat mastitis and of associated inflammatory biomarkers detected in goat milk will follow a summary of our proteomic investigations of goat milk during the course of experimentally-induced mastitis. Moreover, the phosphorylation of caprine casein proteins and their potential implications as markers of disease will be discussed.

2. Proteomics approaches

The proteomic field is divided into two main analytical methodologies: the top-down and the bottom-up. The top-down approach relies on the analysis of intact proteins and corresponding fragmentation within the MS. In contrast, the bottom-up approach, which has been increasingly adopted by the proteomics community, proceeds through analysis of peptides that are generated outside the MS. The identified peptide sequences are then reassigned to the proteins they originate from, through database searching. To reduce sample complexities, a protein mixture can also be fractionated by gel-based approaches prior to MS analysis. The spots from the gel are excised and are subjected to an in-gel digestion. In the following sections, gel electrophoresis, MS, and database searching will be briefly discussed.

2.1. Electrophoresis

Classical proteomic approaches take advantage of protein fractionation by using gel-based assays. 1D-SDS/PAGE and two-dimensional electrophoresis (2-DE) have provided direct separation technologies and contributed to the better understanding of the global milk proteome. 2-DE involves separation by isoelectric focusing in the first dimension, followed by SDS-PAGE in the second dimension. 1D-SDS/PAGE offers a number of important advantages as it produce sharp, molecular weight-separated bands, which increase the dynamic range of the mixture analysis. Fractionation of the complex mixture by spreading it out over 10–20 gel slices dramatically increases the depth of analysis, and hence the number of identified proteins. 2-DE is useful to optimize the separation of proteins of similar molecular weight but with different isoelectric points, which are not resolved using SDS/PAGE. 2-DE provides higher resolution compared to SDS/PAGE and is the best choice for the analysis of phosphorylated or glycosylated proteins as their isoelectric point can shift. In fact, 2-DE is the only technique that provides a visualization of PTM. On the other hand, low abundance, highly charged and hydrophobic proteins such as membrane proteins cannot be well resolved in

2-DE. 2-DE is often time-consuming and requires sample cleanup prior to analysis. In both 1D- and 2-DE analyses, the bands or spot map are visualized by protein staining with Coomassie. The targeted bands from 1D spots or spots from 2D gels are sequenced and identified by mass spectrometry. In a bovine mastitis study, Boehmer et al. performed an optimized protein separation with 2D electrophoresis to analyze whey from control and mastitic bovines. The experiments were conducted with a conventional proteomic approach using 2-DE as a choice of separation method, coupled by MALDI-TOF analysis for the identification [30].

2.2. Mass spectrometry

Although gel-based assays are useful for fractionating complex mixtures and for removing contaminants from a sample that might interfere with the MS analysis, gel-free approaches are simple and straightforward. Using a gel-free approach, a complex protein mixture is directly digested in solution prior to MS analysis. Reduction and alkylation steps, though not required prior to enzymatic cleavage, provide greater peptide yields and can enhance peptide sequence coverage. Prior to MS analysis, peptides can be fractionated using different forms of chromatography including reverse-phase, strong and weak ion exchange, size exclusion, and affinity capture chromatography. Although reverse-phase chromatography is the most widely used method of LC separation in proteomics, affinity capture chromatography is commonly used to enrich or isolate intact proteins, or to investigate PTMs prior to MS analysis.

There are different types of MS instruments for the analysis of a particular type of sample, each with different scanning speed and resolution capabilities. Nonetheless, all MS instrument systems have three common and distinct components: an ionization source for the generation of ions, a mass analyzer for the separation of ions, and a mass detector for the detection of ions.

In regards to ionization, it is important to note that soft ionization techniques such as MALDI and electrospray ionization (ESI) have revolutionized the analysis of large biomolecules. In MALDI, a sample is mixed with a UV-absorbing crystalline matrix, most commonly derivatives of cinnamic acid, and spotted onto a metal target plate. The plate is then inserted into the MS instrument, where it is placed in a vacuum and irradiated with a UV laser. The matrix absorbs the irradiation, which simultaneously heats, volatilizes, and ionizes the sample [16]. Once ionized, the proteins or peptides are analyzed in a mass analyzer, which is typically time-of-flight (TOF) in a MALDI instrument. The TOF mass analyzer is ideal for MALDI because it has a virtually unlimited mass range, which is advantageous because MALDI yields singly charged molecular ions that can have a high mass-to-charge (m/z) ratio [30]. TOF instruments can also operate in tandem mode, using a second mass analyzer to monitor fragment ions (i.e., TOF/TOF).

In contrast to MALDI, ESI produces multiply charged species for each molecule. This is due to the mechanism of ion formation in ESI in which an electrical field leads to the transfer of ions from a solution into the gaseous phase. The transfer of an ionic species from solution into the gas phase by ESI involves three steps that include the dispersion of charged droplets, followed by solvent evaporation, and finally the ejection of the ion from the highly charged droplets [31]. Although the generation of multiply charged ions complicates the mass analysis, it greatly enhances fragmentation potential, which is necessary for structure elucidation. Ideal

mass analyzers for ESI are single or triple quadrupoles and quadrupole ion traps. Because it is a liquid-based method, ESI is more compatible with the LC separations and has consequently became the standard ionization method in LC-MS/MS experiments. With the advent of ultra-performance liquid chromatography (UPLC), chromatographic separations have improved in terms of both the resolving power and the speed of the separation. Nanoflow LC (nLC) is now also widely used in proteomics because it is amenable to LC columns with a smaller internal diameter, which increases the column backpressure and results in greater sensitivity [32].

2.3. Bioinformatics

The accurate identification of proteins and peptides in a complex mixture using tandem mass spectrometry, data can be achieved by database searching strategies. The databases are normally protein sequences translated from genomic data. Considering a database containing potential proteins that could be present in the sample, these proteins are digested in silico by a search engine. For example, for tryptic peptides, the search engine would calculate the masses of all peptides that could be produced by cleavages after lysine and arginine residues and would create a virtual peptide database [33]. Similarly, for the identification of peptides in the sample, the search engine first filters all potential peptides with the same mass, then performs an in silico fragmentation of each of these peptides. After matching peptide sequences with the list of fragment masses observed in the MS/MS spectrum, the search engine assigns a score [33].

The success of MS-based proteomics analyses depends on the availability of complete and accurately annotated databases containing the gene and protein sequence information for the animal species of interest. Unfortunately, as of yet, only a limited number of annotated genome sequences are available for the goat. In recent years, despite an increase in the number of proteomic investigations of various body fluids from the goat, identifying these proteins is recognized as a greater technical challenge [34]. In the absence of annotated protein databases, researchers are forced to extrapolate their data from the bovine genome or other mammalian protein databases [28]. If a given database does not contain the amino acid sequences of all of the proteins in the sample, suitable matches cannot be made; thus the proteins will remain unidentified.

3. Proteomic research in caprine milk

Milk is composed of three main components: casein, whey proteins, and milk fat globule membrane (MFGM). Using differential centrifugation and ultracentrifugation, it is possible to isolate these three fractions. While the application of proteomics in the milk of larger animals has attracted many research groups, the role of proteomics in the milk of goat remained limited. In recent years, with the advancements of available technologies in proteomics, there has been a growing interest to unravel the dynamic structure of goat milk protein contents. Proteomic research in caprine milk has been applied in the areas of identifying major proteins, comprehensive analysis, MFGM, and identification of PTM (**Table 1**).

	Techniques	Matrix	Proteomic study	References[1]
Major proteins	2-DE & MALDI-TOF	Milk	Casein profile	[24]
	nLC-MS/MS	Cheese	Comparative proteomics/ adulteration	[35]
	1-DE & LC-MS/MS	Skim milk	Comparative proteomics	[27]
	MALDI-TOF	Raw milk	Comparative proteomics	[25]
	2-DE & MALDI-TOF	Skim milk	Farm animals principle proteins	[36]
	2-DE & nLC-MS/MS	Skim milk	Comparison of healthy and LPS induced	[28]
	1DE/2-DE/HPLC	Skim milk	Comparative proteomics Brazilian breeds	[37]
	MALDI-TOF	Skim milk	Anti-inflammatory/anti-allergic properties	[3]
	MALDI-TOF-MS	Skim milk	Fingerprinting of principle proteins	[26]
	MALDI-TOF-MS	Skim milk	Fingerprinting of major proteins/adulteration	[38]
	2-DE/ELISA	Colostrum/skim milk	IgG/IgM bindings profile & quantification	[39]
	UPLC-XevoTQS	Milk powder	Absolute quantification (MRM) Whey powder	[40]
Comprehensive	SCX & nLC-MS/MS	Whey	Farm animals comparative & quantification	[41]
	CPLL, 1DE & nLC-MS/MS	Milk	Comprehensive of low abundance proteins	[6]
	2-DE & MALDI-TOF & LC-MS/MS	Milk	Comparison study sheep and goat	[42]
MGFM	1D SDS/PAGE	MFGM	Protein composition of MFGM	[43]
	1DE &MALD-TOF	MFGM	Assessment of protein composition	[8]
	LC-MS/MS	MFGM	Proteome profile and biological activity	[44]
	nLC-MS/MS	MFGM	Colostrum & mature milk	[45]
	2-DE/nLC-MS/MS	MFGM	Quantification of mammalian	[46]

	Techniques	Matrix	Proteomic study	References[1]
PTM	1DE & nLC-MS/MS	Skim milk	Comprehensive caseins phosphoproteome	[29]
	Affinity chromatography & nLC-Chip-QTOF-MS	Skim milk	Lactoferrin N-glycans in human and bovine	[47]
	nLC-MS/MS	MFGM	N-glycosylation comparison in mammals	[46]
	LC-MS/MS	MFGM	Phosphoproteome analysis	[48]

[1]Literature references of each study: 2-DE, two-dimensional; 1-DE, one-dimensional; nLC, nano liquid chromatography; UPLC, ultra-performance liquid chromatography; SCX, strong cation exchange; CPLL, combinatorial peptide ligand libraries; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; MRM, multiple-reaction-monitoring.

Table 1. Proteomic investigations of caprine milk.

3.1. Identifying major proteins

One of the first proteomic studies in goat milk was reported by Roncada et al., where they used two dimensional electrophoresis (2-DE) combined with MALDI-TOF and ESI-ion trap mass spectrometers to analyze α_{S1}-casein alleles. They determined casein polymorphisms as the key characteristics in the cheese manufacturing industry [24]. Major goat milk proteins including caseins (α_{S1}, α_{S2}, β-, and κ-casein) and some of the whey proteins (albumin, lactoferrin, β-lactoglobulin, and α-lactalbumin) are highly abundant and have been widely studied [8, 25, 37]. Several groups compared the data from proteomics studies of goat milk with those of cow and other species, but their reports involved only the characterization of major proteins [27, 36, 41]. In one of 2-DE analyses [36], highly abundant proteins in each animal species display their own unique pattern [36]. This group further highlighted significant interspecies differences in milk from different ruminants and identified β-lactoglobulin as the major whey protein in many ruminants including goat.

The use of goat milk as a substitute for cow milk for allergic people has been also recently reported [3, 10]. In some parts of the world, milk of donkey or goat is used in newborn and infant feeding because they are less allergenic than cow milk. Accordingly, Di Girolamo et al. used fingerprinting of major milk proteins by MALDI-TOF MS coupled to a robust statistical analysis to determine adulteration and unintended contamination of donkey milk and goat milk [26]. Peptide mass fingerprinting is a simple methodology in proteomics where proteins are cleaved with a protease, such as trypsin. The identification is accomplished by matching the observed peptide masses from MS data to the theoretical masses derived from a sequence database [49]. Comparative proteomic approaches were used to study colostrum and milk of goats, cows, and sheep to determine chemical composition and immunoglobulin concentration [39]. The study revealed that despite the similar immunoglobulin concentrations in colostrum and milk from the three studied species, differences in several immune components can be detected.

Proteomics is instrumental in detecting milk adulteration as adulteration has been a significant problem in the dairy industry. In a comparative proteomic study, different cheese samples obtained from milk of cow, sheep, and goat were analyzed using HPLC-chip-MS/MS [35]. The authors found κ-casein to have a unique primary structure and suggested that it could be used to determine the origin of milk in different cheese samples. In another study, a MALDI-TOF-MS platform was used to profile milk samples for the rapid detection of illegal adulterations caused by the addition of either nondeclared cow milk, milk of other species, or the addition of powdered milk to the fresh counterpart [38]. For this purpose, peptide and protein markers of cow, water buffalo, goat, and sheep milk were identified and the effects of thermal treatment-associated adulterated milk samples were evaluated. This study introduced an independent, complementary peptide profiling measurement and extended proteomic approaches to the analysis of thermal treatment. Yang et al. analyzed milk whey samples obtained from a number of species including goat, cow, buffalo, yak, and camel. They detected certain proteins as the characterizing traits for a given species that could be used to evaluate adulteration [41].

3.2. Comprehensive analysis

Proteomic investigation is challenging due to the wide dynamic range of protein expression where the presence of high abundance protein masks or prevents the detection of low abundance proteins. Recently, the most comprehensive proteomic dataset of goat milk has been reported by Cunsolo et al. [6]. This group fractionated the total milk samples using combinatorial hexapeptide ligand libraries (CPLL; such as ProteoMiner) at different pH levels to reduce the dynamic range of protein concentrations. They identified 452 unique gene products including many low abundance proteins in goat milk. Their success was also related to the use of further fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), high resolution, nLC-MS/MS, and an advanced bioinformatics platform [6]. New strategies employing multi-enzyme digestion coupled with CID and ETD for protein sequencing and characterization was also used to increase sequence coverage and localization of PTMs [50]. In a separate study, using two complementary proteomic approaches, Anagnostopoulos et al. investigated the milk whey of three Greek sheep and goat breeds. They identified about 600 protein groups, most of which were involved in nutrient transport and immune system responses [42]. These findings provide the most comprehensive description of the goat milk proteome that can be utilized to build a goat protein sequence database. As it was pointed out by Soares et al. the success of proteomic-based investigations largely depend on the availability of complete and annotated databases containing the gene and protein sequence information for different animal species [51]. Hence, the data generated from these recent, more comprehensive proteomics analysis of the goat milk proteome could facilitate the detection and characterization of additional milk proteins in future proteomics analyses of goat milk.

While proteomic analyses have been used to qualitatively identify adulterants in milk, no reliable, selective, and sensitive method existed to obtain absolute quantification. In a clever analysis, Chen et al. used multiple reaction monitoring (MRM) to quantify milk adulteration

when cow milk was added to sheep or goat milk [40]. In this study, two peptides derived from β-lactoglobulin were chosen as the protein markers. Similar isotopically labeled peptides as internal standards were designed and synthesized to minimize the matrix effect, which led to more accurate quantification results. MRM has been used for many years to measure and quantify small molecules, drugs, and metabolites. However, the application of MRM to obtain absolute quantitation of proteins is relatively new and offers great potential to the field.

3.3. Analysis of MFGM

Goat milk is a great source of essential fatty acids, which are concentrated in MFGM along with a complex mixture of proteins, glycoproteins, and enzymes [52]. Although MFGM proteins account for a small fraction of the total milk protein, they have been extensively studied in human and bovine milk. In previous bovine MFGM studies, many health benefits including anticancer, antimicrobial, and antiviral effects have been attributed to the glycoproteins [53]. Therefore, there is a need to characterize this important component in goat milk.

The protein composition of goat MFGM has been described by several groups [8, 43, 44, 53]. Cebo et al. used 1DE and MALDI-TOF-MS and reported butyrophilin, lactadherin, mucin, and lectin as major MFGM proteins in goat milk. Interestingly, lactadherin from goat milk consisted of a single polypeptide chain whereas 2 polypeptide chains were detected in bovine milk [8]. In addition, the MFGM of colostrum and mature milk was also investigated [45]. As expected, the acute phase proteins were higher in colostrum MFGM, signifying the importance of colostrum intake to the immune system of newborns.

In a more recent 2016 study by Yang et al., the N-glycoproteome of MFGMs, obtained from a group of mammals' milk including human, Holstein, Jersey, buffalo, yak, camel, horse, and goat was investigated. They found that protein components of MFGM fractions from ruminants were more similar to each other when compared to nonruminants [46]. In a comprehensive analysis of goat milk MFGM, Henry et al. reported the use of high-resolution LC-MS/MS to expand the MFGM proteome in goat milk to 442 functional groups [48]. The main focus of their study was to probe the phosphoproteome of goat MFGM that will be covered in the next section.

3.4. Post-translational modifications (PTM)

PTMs are chemical modifications that play a key role in functional proteomics. The characterization of PTMs, although challenging, is very important as they regulate protein function and control numerous important biological processes. A large number of different PTMs have been reported, but by far, phosphorylation and glycosylation are the most important and well-studied [54]. Since phosphorylation has a low stoichiometry, there is a need for enrichment of phosphopeptides in an analysis of a complex mixture. The current method to enrich phosphopeptides is based on affinity purification using phosphospecific antibodies immobilized-metal affinity chromatography (IMAC). Zhong et al. optimized the selective isolation of mono- and multi-phosphorylated peptides by using different forms of iron ions [55].

In this study, they selected α-casein and two synthesized mono- and di-phosphopeptides as a model system to demonstrate that $NiZnFe_2O_4$ was highly selective for multi-phosphopeptides whereas Fe_3O_4, $NiFe_2O_4$, and $ZnFe_2O_4$ had a higher affinity for mono-phosphopeptides. Along with the improvements of IMAC for phosphoproteomic experiments, instrument enhancements including improved acquisition speed allowed the identification of many more phosphopeptides per analysis. As mentioned before, in a comprehensive analysis of goat milk MFGM phosphoproteome, Henry et al. used TiO_2 for enrichment of the MFGM samples. Using nLC-MS/MS and high resolution mass spectrometer, they characterized the phosphorylation of several key mammary gland proteins in goat MFGM. This group, leveraging the strengths of high resolution and faster acquisition time, reported the detection of 271 sites of phosphorylation on 124 unique goat MFGM proteins [48].

The identification of PTMs is especially useful for the detection and characterization of acute phase proteins (APPs) during disease because APPs are subjected to modification. The N-glycan profiles of goat milk lactoferrin were compared with human and bovine milk using advanced mass spectrometry techniques [47]. The characterization of glycan composition established high mannose, hybrid, and complex N-glycans. Among the N-glycan compositions, 37% were sialylated and 34% were fucosylated. This group highlighted the existence of similar glycan composition between human and goat milk and discovered a novel glycan in goat milk that was not detected in human milk. A recent 2016 study investigated N-glycoproteome analysis of MFGMs from a number of mammals' milk [46]. They observed different glycosylation patterns in certain proteins that were previously reported with varying molecular weights based on the analysis by SDS-PAGE. They concluded that these discrepancies were the result of the differences in carbohydrate content of these proteins.

4. Mastitis and inflammatory-related biomarkers in goats

Mastitis is inflammation of the mammary gland that manifests in a wide range of physical indications, chemical changes in the milk, and pathological changes in the udder [56]. Clinical symptoms of mastitis include swelling and pain in the udder, increased rectal temperature, reduced feed intake and milk production, and the watery appearance or presence of clots in the milk. Mastitis research has drawn immense interest over the years because of its profound economic impact on the dairy industry. The incidence of clinical mastitis can be reduced by the application of management strategies, including a greater awareness of efficient milking and hygienic measures. However, despite the development of vaccines and other preventative methods, mastitis caused by Gram negative environmental pathogens and coliform bacteria remain problematic to treat and to manage [57]. Coliform bacteria are normal inhabitants of soil, manure, bedding, and water; thus, coliform mastitis can occur due to the contact of teats with the infected environment. Although innate immune responses in the mammary gland are effective to some extent, the mammary gland defense mechanisms can be compromised by environmental and physiological conditions [58]. Despite extensive knowledge of the bovine host response to mastitis pathogens and the effects of mastitis infection on the

bovine milk proteome, only a limited understanding of the goat innate immune response to mastitis pathogens or the subsequent changes in goat milk protein expression over the course of a clinical infection exists. Nonetheless, like other ruminants that are managed for milk production, goats are also susceptible to and affected by mastitis.

Soluble mediators of inflammation in bovine milk and plasma during clinical mastitis have been studied extensively using antibody-based strategies [59]. Although antibody-based methodologies are both quantitative and accurate, they have limited detection capabilities. Conversely, mass spectrometric-based proteomic technologies allow for the simultaneous analysis of a larger number of proteins without the reliance on antibodies. Using MS-based proteomics, a number of biomarkers including APPs were identified in bovine serum and milk, which were correlated with pain and disease status [19, 60]. The concentration of most APPs typically increased during infection or inflammation, and the increased levels were relatively stable and persisted for a number of days, or even weeks, after the original insult or stimulus [19]. Despite the fact that our knowledge of the modulation of the bovine milk proteome during mastitis continues to expand, very little comparative data exists on lactating dairy goats.

In regards to the study of the goat milk proteome, our group detected increases in haptoglobin (Hp), serum amyloid A (SAA), and lactoferrin in the milk of goats following an intramammary infusion of lipopolysaccharide (LPS) to induce coliform mastitis [28]. Other studies also documented significantly increased blood levels of Hp and SAA in an experimentally induced subacute ruminal acidosis in goats [61]. The majority of APPs are known to be glycosylated. Due to the high extent of its carbohydrate moiety, the APP alpha-1-acid glycoprotein (AGP) has been established as a biomarker of inflammation in goats [62]. AGP was also reported to potentially inhibit neutrophil migration to the site of infection, leading to inadequate bacterial clearance and resulting in increased risk of mortality [63]. Further, Heller et al. determined the species-specific reference intervals for four APPs including Hp, SAA, AGP, and lipopolysaccharide-binding protein (LBP), which is a soluble polypeptide that binds to bacterial LPS and increases its proinflammatory activity up to 1000 fold, in goat milk [64, 65].

4.1. Effects of experimentally-induced mastitis on the goat proteome

Modulations in the expression of goat milk proteins have been examined following an experimental induction of endotoxin mastitis by intra-mammary infusion with LPS. For details of challenge study and sample preparation, see materials and methods section in Ref. [28]. Crude milk samples were separated by 2DE prior to nLC-MS/MS analysis. The unique proteins identified following the 2DE analysis of skim milk from healthy goats and skim milk collected from the same goats 18 h post infusion with LPS are summarized in **Table 2**. In the absence of goat specific database, we used the Swiss-Prot other mammalia taxonomy, which includes only a limited number of goat sequences. Though some goat specific proteins were identified, the majority of the protein identifications were assigned to other species. As shown in this table, caseins constitute the most abundant proteins in milk; thus, a marked number of casein variants, specifically β- and α_{S2}-caseins, which were detected in 13 and 6 separate

Protein ID	Protein name	Species[1]	Peptides[2]	Sample[3]
Q28372	Gelsolin	Equine	2	Healthy
Q3SX14	Gelsolin	Bovine	6	LPS
P85295	Serum albumin	Caprine	6	Healthy
P14639	Serum albumin	Ovine	9	LPS
P18626	α_{S1}-Casein	Caprine	12	Healthy
P18626	α_{S1}-Casein	Caprine	15	LPS
P11839	β-Casein	Ovine	3	Healthy
P11839	β-Casein	Ovine	4	LPS
P04654	α_{S2}-Casein	Ovine	4	Healthy
P33049	α_{S2}-Casein	Caprine	5	LPS
P02670	κ-Casein	Caprine	6	Healthy
P02670	κ-Casein	Caprine	6	LPS
P02756	β-Lactoglobulin	Caprine	22	Healthy
P02756	β-Lactoglobulin	Caprine	10	LPS
P02694	Retinol-binding protein-1	Bovine	4	Healthy
P02694	Retinol-binding protein-1	Bovine	2	LPS
P00712	α-Lactalbumin	Caprine	3	Healthy
P00711	α-Lactalbumin	Bovine	2	LPS
Q4TZH2	Fatty acid-binding protein	Bovine	5	Healthy
Q6QAT4	β-2-microglobulin	Ovine	3	healthy
Q6QAT4	β-2-microglobulin	Bovine	4	LPS
Q29477	Lactotransferrin	Caprine	19	LPS
Q32PJ2	Apolipoprotein A-IV	Bovine	9	LPS
B6E141	Haptoglobin	Ibex	3	LPS

Protein ID	Protein name	Species[1]	Peptides[2]	Sample[3]
P19661	Cathelicidin-3	Bovine	2	LPS
P22226	Cathelicidin-1	Bovine	3	LPS
P42819	Serum amyloid A	Ovine	4	LPS
P02584	Profilin-1	Bovine	2	LPS

[1]Species of highest scoring assignment from Swiss-Prot (http://www.uniprot.org/).
[2]Number of peptide assignments.
[3]Samples were either obtained from healthy goats or induced by LPS.

Table 2. Proteins detected in milk of healthy goats and experimentally induced with endotoxin mastitis (LPS).

spots at varying isoelectric points on the gel, respectively, were observed in the milk of the goats prior to LPS infusion. The presence of full lengths β- and α_{S2}-casein and in several spots of varying mass and isoelectric charges was most likely due to the presence of multiple fragments of the two dominant caseins in the skim milk as a result of proteolysis. Conversely, the α_{S1}- and κ-caseins were detected in only two spots on the gel. Similar to the protein expression profiles generated from milk samples collected prior to infection, the β- and α_{S2}-caseins dominated the profiles of the skim milk samples collected 18 h following LPS infusion. The number of β-casein fragments at lower-MW detected on the gel was reduced, but the number of α_{S2}-casein spots, both full length protein and corresponding fragments increased in the 18 h samples. In addition to the caseins and the whey proteins β-lactoglobulin and α-lactalbumin, the lower abundance proteins serum albumin, gelsolin, retinol binding protein, fatty acid binding protein, and β-2-microglobulin were likewise detected in goat skim milk samples collected just prior to challenge with LPS.

In sharp contrast to prior reports of bovine milk protein profiles during coliform mastitis, vascular-derived proteins such as complement factors and serotransferrin (known to leak into bovine milk following cytokine induction and the subsequent breakdown of the blood-milk barrier) were not detected in our analysis. Similarly, although serum albumin was detected, no significant increase in the abundance of the vascular-derived protein was apparent. However, along with the increases in SCC, several proteins with antimicrobial properties that are known to be found in the granules of neutrophils, the primary component of SCC during mammary infections, were detected in the goat skim milk samples at 18 h after induction of endotoxin mastitis including cathelicidin-1 and cathelicidin-3 and lactoferrin. The induction of the acute phase response during endotoxin mastitis in goats was also apparent as the APP haptoglobin (Hp) and SAA were likewise detected in the mastitic goat skim milk samples collected 18 h after intra-mammary infusion with LPS.

The intensity of the spot corresponding to serum albumin remained the same in the gels of the goat milk samples 18 h post challenge, which could indicate that the breakdown of the blood-milk barrier during endotoxin mastitis might not be as profound in goats as has been observed in dairy cattle. The inflammatory response was however, supported by elevated

SCC in the goat milk following inoculation with endotoxin, as well as by the presence of both antimicrobial and APPs. The results likewise provided preliminary information regarding protein modulations of goat milk during disease as well as added knowledge of the host response during endotoxin mastitis in goats [28].

4.2. Analysis of multiphosphorylation sites in caseins

Caseins exist in four different variants including α_{S1}-, α_{S2}, β-, and κ-casein. Despite little homology, there is a rare conserved sequence (SSSEE) present in α_{S1}-, α_{S2}, and β-casein that serves as a multiphosphorylation site [66]. This conserved sequence motif does not exist in κ-casein, although it does possess two phosphorylation sites embedded in the C-terminus of the protein. The existence of the conserved sequence domain in casein multi-phosphopeptides makes their detection more challenging as the two glutamic acid (E) residues further increase their hydrophilic nature. In the presence of other peptides, they often go undetected by mass spectrometry. Thus, a vast majority of researchers use IMAC to enrich multi-phosphopeptides prior to detection. Without using any enrichment strategies, we reported changes in the levels of caprine casein phosphorylation [29].

In our experiments, we used milk samples obtained from healthy goats before and after experimental induction of endotoxin mastitis with LPS. We isolated casein bands using 1D-SDS/PAGE prior to in-gel digestion and analysis by nLC-MS/MS. Despite their large size, the majority of these tryptic phosphopeptides eluted early during chromatographic separation. As well, many were not detected following database searching or were assigned a very low peptide score. Consequently, manual inspections of the MS/MS spectra were necessary to validate the identifications.

In addition to mono- and di-phosphorylation sites, a number of multiphosphorylation sites exist in α_{S1}-, α_{S2}-, and β-casein (**Figure 1**). As shown, the conserved SSSEE domain forms a hexa-phosphopeptide in α_{S1}-casein, a tetra-phosphopeptide in β-casein, and two different multi-phosphopeptides in α_{S2}-casein. We characterized 18 different phosphorylation sites from a series of mono- and multi-phosphopeptides.

Examples of multi-phosphopeptides detected in our analysis are presented in **Figure 2**. The top MS/MS spectrum corresponds to a di-phosphopeptide (D58−K73) in α_{S1}-casein that was detected as a doubly charged ion (MH^{2+}= 951.32) corresponding to (MH^{+} = 1901.64, **Figure 2A**). In the α_{S2}-casein, a triply charged ion (MH^{3+} = 1039.89) with the monoisotopic mass (MH^{+} = 3117.08) was isolated and fragmented in a linear ion trap to produce the MS/MS spectrum as shown in **Figure 2B**. The bottom MS/MS spectrum corresponds to the tetra-phosphopeptide (E17−K43) that was detected in β-casein as a triply charged ion (MH^{3+} = 1103.10) corresponding to (MH^{+} = 3307.29, **Figure 2C**). The phosphorylation sites were accurately assigned in each multi-phosphopeptide despite the presence of multiple other serine and threonine resides. In α_{S1}-casein, we did not detect the hexa-phosphopeptide, which also contains the conserved domain. Likely, the proximity of numerous phosphorylated sites made it even more fragile. Nevertheless, we clearly characterized one mono- and one di-phosphopeptide in α_{S1}-casein [29].

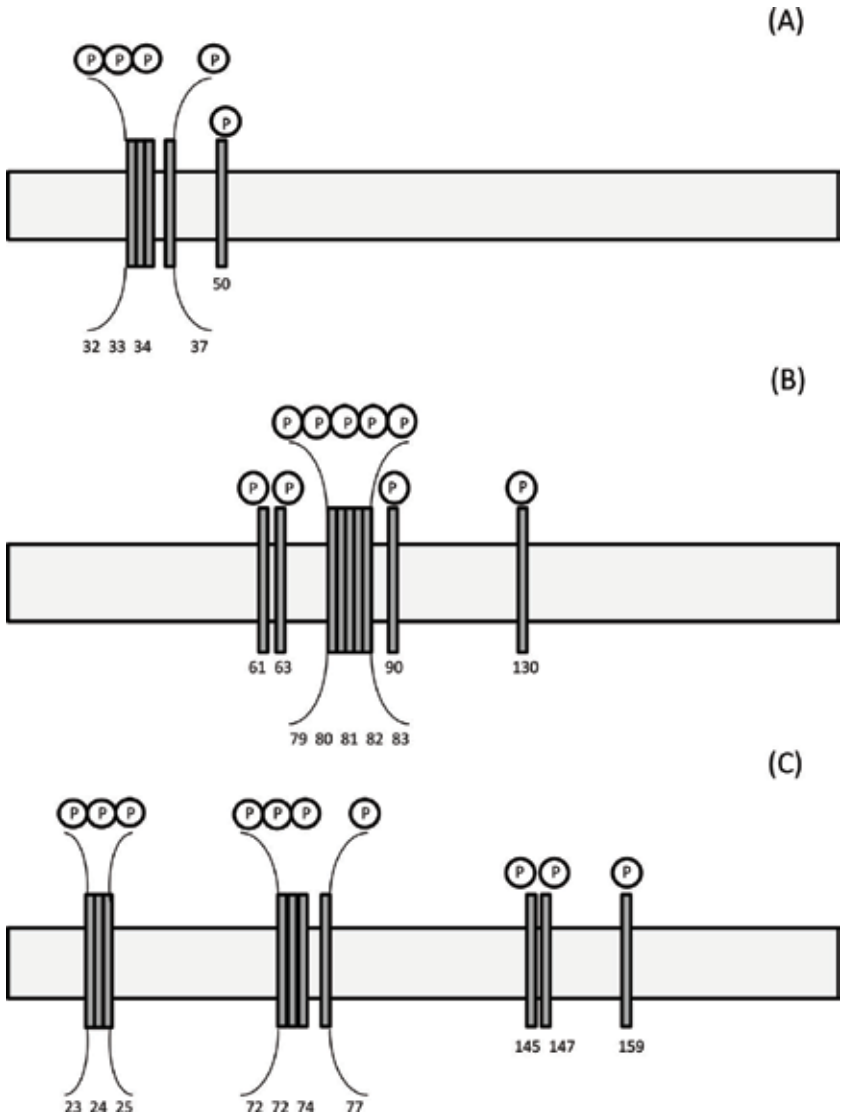

Figure 1. Identification of potential serine and threonine phosphorylation sites within β-casein (A), α_{S1}-casein (B), and α_{S2}-casein (C). The conserved sequence domains (SSSEE) that serve as the multiphosphorylation sites are present in all three caseins.

Despite the lower apparent abundance, the multi-phosphopeptides shown in **Figure 2** were also detected in milk samples obtained from animals with experimentally-induced endotoxin mastitis. In α_{S2}-casein, we also detected a tetra-phosphopeptide (N62–K86) with the amino acid sequence NANEEEYSIRSSSEESAEVAPEEIK [29]. The phosphorylation sites were found at S72, S73, S74, and S77. However, this multi-phosphopeptide was never observed in the mastitic goat milk samples. Instead, this ion was isolated and fragmented in the linear ion trap in which we readily detected two unmodified peptides. The first tryptic

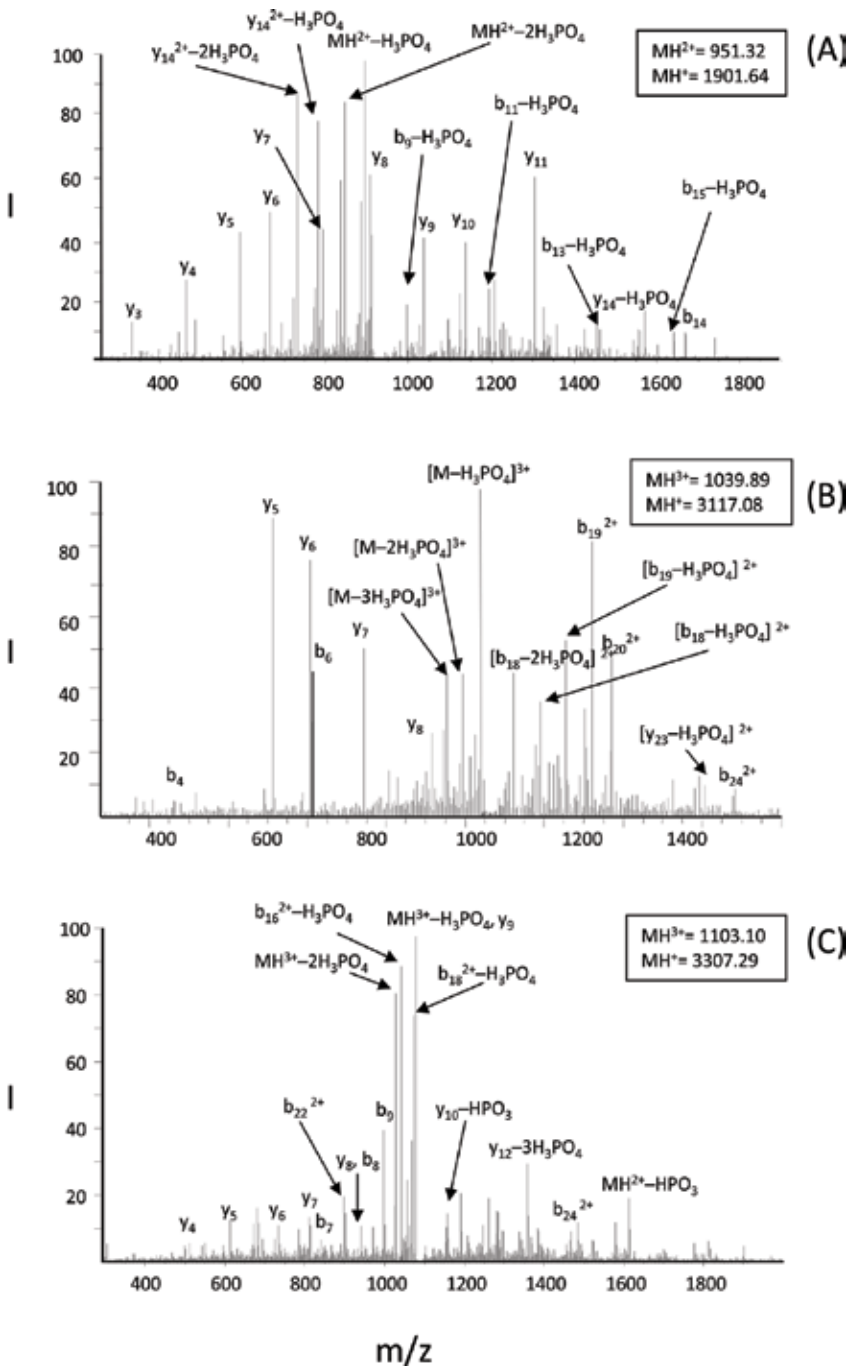

Figure 2. Tandem mass spectra of multi-phosphopeptides: (A) di-phosphopeptide in α_{S1}-casein, (B) tetra-phosphopeptide α_{S2}-casein, and (C) tetra-phosphopeptide β-casein. The *y*- and *b*-ions are marked in each spectrum with losses of phosphoric acid (neutral loss) probably owing to an in-source fragmentation.

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peptide in the amino acid sequence (NANEEEYSIR) was detected as an intense doubly charged ion (MH^{2+} = 614.18 Da) corresponding to (MH^{+} = 1227.36 Da). The second dephosphorylated segment (SSSEESAEVAPEEIK) was detected as a doubly charged ion (MH^{2+} = 796.68 Da) corresponding to (MH^{+} = 1592.36 Da). These observations clearly indicated that the peptide remained dephosphorylated in mastitic goat milk. The attachment of multiple phosphate moieties might have sterically hindered the trypsin cleavage site and as a result, we observed the tetra-phosphopeptide as a missed cleavage. To this end, it has been reported that the proximity of the cleavage sites to the phosphorylated amino acids could impair tryptic digestion [67].

5. Conclusions

Advances in separation and mass spectrometry capabilities, enable our abilities to identify and characterize proteins and their PTMs. This chapter provides an overview of proteomics investigations in goat milk, from identifying major milk proteins to comprehensive analysis in different fractions and PTMs. Many challenges still exist, but technological advances have led to an increased in research contributing to a better understanding of the proteomic analysis of goat milk. Low-abundance proteins and disease-specific proteins have been identified as potential biomarkers. Using proteomics strategies, the efforts of our group and others have shed some light on the role of APPs during coliform mastitis and other diseases in goats. The host response during infection and related changes in the goat milk proteome remains comparatively limited. Nonetheless, our comparative proteomic analysis suggests that the caprine host response to endotoxin could differ from other ruminant species. Finally, our precise characterization of casein phosphorylation in goat milk before and after challenge with LPS offers new insights into protein modulations in goat milk during mastitis.

Disclaimer

The views expressed in this article are those of the author do not necessarily reflect the official policy of the Department of Health Human Services, the U.S. Food Drug Administration, or the U.S. Government.

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Chapter 9

Development of an *In Vitro* Goat Mammary Gland Model: Establishment, Characterization, and Applications of Primary Goat Mammary Cell Cultures

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Additional information is available at the end of the chapter

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Abstract

Alternatives to animal experiments, based on *in vitro* methodologies, have been suggested and adopted in the last decades in order to completely substitute or to reduce animal numbers in *in vivo* assays. In this chapter we describe methods for establishment, maintenance, and characterization of primary goat mammary epithelial cell cultures (pgMECs) and possible applications for which the derived primary cell model can be used instead of *in vivo* experiments. The established cell lines were grown *in vitro* for several passages and remained hormone and immune responsive and capable of milk protein synthesis. Knowledge on goat mammary cells and their manipulation is applicable to different fields of research; for example, it could be used in basic research to study mammary development and lactation biology, in agriculture to enhance lactation yield and persistency or to produce milk with special characteristics, in biopharma to express recombinant proteins in goat milk, or in biomedicine to study lactation, mammary development, and pathology, including neoplasia. The established cells represent an adequate surrogate for mammary gland; were successfully used to study mammary gland immunity, lactation, and mammary stem/progenitor cells; and have a potential to be used for other purposes.

Keywords: goat, mammary gland, cell culture, mammary epithelial cell, lactation, mastitis

1. Introduction

Goats are one of the oldest domesticated species. They are bred for milk and meat and play an important role in human nutrition, especially in developing countries. Their number is constantly increasing through the years and the population has been estimated to over a billion

(FAOSTAT, 2013). However, goats are not useful only for food production. Because of their anatomical and physiological characteristics, relatively short gestation period, early sexual maturation, and inexpensive and simple maintenance, they are valuable for basic research, for biotechnology applications, and as animal models in medical research. For example, goats are used to study heart and joint diseases [1] and are an excellent model species to study mammary development and lactation [2].

Rodent mammary gland is the most widely studied and has provided many biological insights, but its anatomy and physiology are not fully representative of human or ruminant mammary gland. Morphological development of mammary gland is much more alike between humans and ruminants [3]. Considering the size, arrangement of the mammary gland (two main glands), and mechanism of secretion, which is apocrine in goats and humans, whereas merocrine in bovine, goats seem to be a better choice for modeling human mammary gland, compared to cows or rodents. Goat mammary tissue/cell cultures can serve as valuable models to study lactation, mammary development, and pathology, including neoplasia, which is for unknown reasons extremely rare in ruminants, despite the anatomical and physiological similarities to humans [4]. Additionally, genetically modified ruminants (especially goats) have been used as "bioreactors" for production of recombinant proteins. Recombinant proteins can be controlled by inducible, mammary-specific promotors and expressed in mammary gland, from where they can be relatively easily isolated from milk. For example, the first marketed human recombinant protein produced in transgenic animals was produced and extracted from milk of transgenic goats [5]. Furthermore, transgenic dairy goats can be used for production of milk with special nutritional characteristics [6], which can be beneficial especially in developing countries.

Knowledge on goat mammary cells and goat mammary biology is beneficial to different fields of science, for example, agriculture (enhancing lactation yield and persistency, and producing milk with special characteristics), basic research (understanding mammary biology), medicine (model organisms), and biopharma (expression of recombinant proteins in goat's milk). In this chapter, we describe methods for establishment and characterization of primary goat mammary cell cultures (pgMECs) and possible applications for which the cell model can be used instead of the mammary tissue. The established cells can be grown *in vitro* for several passages and remain hormone responsive and capable of milk protein synthesis. The cells can be used for basic research of lactation biology, mammary gland immunity studies, mammary stem/progenitor cell identification/isolation, and further applications.

2. Materials and methods

In this section, we briefly describe materials and methods used for establishment, growth, characterization, and procedures with the primary cultures, which apply to the results described in the successive sections.

2.1. Establishment and maintenance of the primary goat mammary epithelial cells (pgMECs)

2.1.1. Tissue processing

Primary cultures were established from mammary tissue of slaughter animals. Lactating goats of different age and nonlactating juvenile goats from approximately four to seven months of age were used for tissue collection. The whole mammary gland was removed immediately after slaughter, wiped with 70% ethanol, and processed under aseptic conditions. First, larger pieces of the glandular tissue were removed from the gland. Alternatively, tissue biopsates can be used instead of whole mammary gland. Different quantities of the tissue can be processed, depending on the desired amount of cells in the culture. In our case, approximately 100 g of the dissected tissue pieces were washed in Hank's balanced salt solution (HBSS), containing penicillin (200 μg/mL), streptomycin (200 μg/mL), gentamicin (200 μg/mL), ampicillin (200 μg/mL), and amphotericin B (10 μg/mL), and mechanically minced with scissors and scalpels. Minced tissue was digested in a 100-ml solution of collagenase and hyaluronidase (400 U/mL of each), prepared in HBSS with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), also containing all of the above listed antibiotics in the same concentrations. The digestion was carried out at 37°C with gentle shaking. Fractions of dissociated cells were collected after 60, 120, and 180 min of incubation, by filtering the contents through a steel mesh and adding fresh solution of the enzymes to the leftovers of the minced tissue. The filtrates were put in 50-ml tubes, washed (diluted) with HBSS, and centrifuged at 1200 rpm for 5 min. The pellets can be resuspended in HBSS and centrifuged several times to remove cell debris. Finally, the resuspended cells were filtered through a 40-μm cell strainer, centrifuged at 1200 rpm for 5 min, resuspended in growth medium, and plated on tissue culture vessels or resuspended in freezing medium (90% FBS and 10% DMSO) for freezing in liquid nitrogen. Major steps of tissue processing are depicted in **Figure 1**.

An alternate to enzymatic digestion, explant culture method is possible. In this case, it is important to mechanically mince the extracted tissue to very small pieces and incubate the finely minced tissue in growth vessels (supplied with growth medium) for several days, using conditions as described hereinafter. After several days, cells will start to explant and attach to culture dishes. Afterward, the tissue pieces can be removed from the vessels and the attached cells passaged into a new dish. To our experience, explant culture will produce lower cell yields; however, the obtained culture might be more enriched in a desired (epithelial) cell type(s) as in the case of enzymatic digestion, where other cell types (e.g. fibroblasts) might be present in a significant amount.

2.1.2. Maintaining pgMECs in cell culture

The cells were grown in RPMI 1640 growth medium, supplemented with 0.1 mM L-methionine, 0.4 mM L-lysine, 2 g/l $NaHCO_3$, 1 mM Na-pyruvate, 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. For simulation of lactogenic

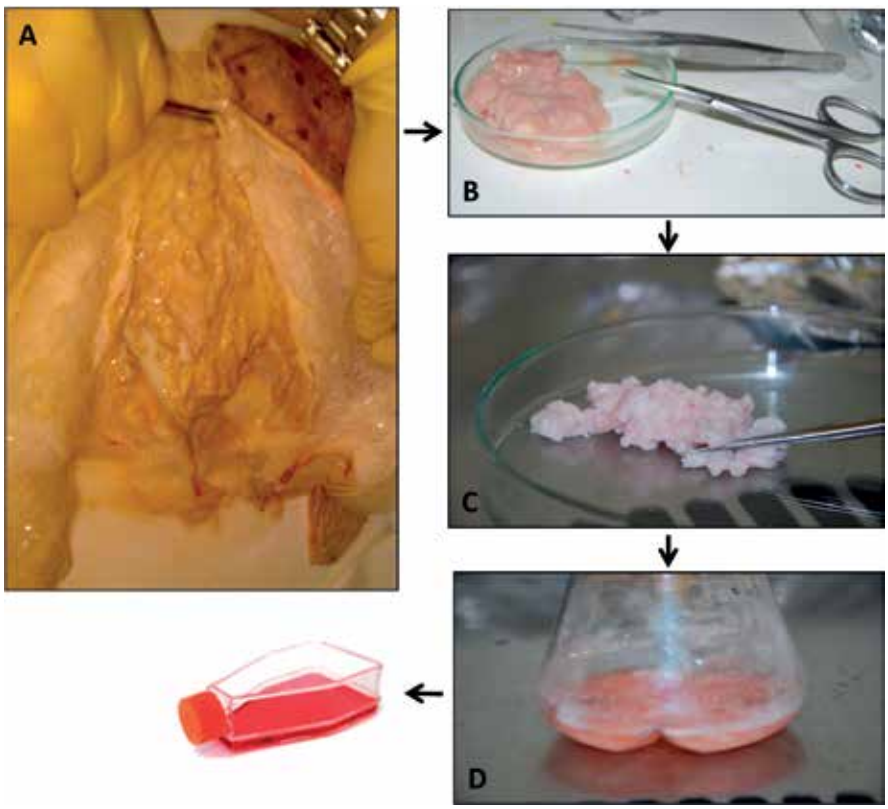

Figure 1. Mechanical and enzymatic processing of the mammary tissue (photo: J. Ogorevc). (A) Removal of the skin covering the mammary tissue. (B) Tissue pieces excised from the gland. (C) Fine mechanical processing of the tissue. (D) Dissociation of the tissue in the cocktail of enzymes. Finally, dissociated cells were collect by centrifugation and seeded in cell culture flasks.

conditions, basic growth medium was supplemented with lactogenic hormones such as insulin (1 μg/mL), hydrocortisone (1 μg/mL), and prolactin (1 μg/mL), and the cells were grown on a commercially prepared basement membrane matrix-covered surface (e.g. Matrigel, Geltrex). The cells were allowed to overgrow the surface, differentiate, and establish cell-cell and cell-surface interactions.

Cells were grown in a 5% CO_2 atmosphere at 37°C, 5% CO_2, and saturated humidity. Growth medium was changed every two to three days. When performing passaging, the cells were treated with 0.25% trypsin-EDTA and incubated at 37°C until the cells detached from the surface. The cells were centrifuged and resuspended in growth medium in a 1:5 ratio. In case of fibroblast contamination, different detachment times of fibroblasts and epithelial cells can be exploited for refining the culture. Fibroblasts form weaker cell-cell and cell-surface interactions and normally detach faster. This characteristic can be used for enrichment of epithelial cells in the culture, using principle of differential trypsinization. Trypsin-EDTA can be diluted to a lower concentration (e.g. 0.05%) to extend detachment times for better control of the procedure.

The established cells should be routinely screened for possible infections with different mycoplasma species. We suggest PCR-based detection of mycoplasma-specific DNA sequences, using 16S ribosomal RNA universal primers as described previously [7].

2.2. Characterization of the pgMECs

2.2.1. mRNA expression of pgMEC-specific markers

For transcription profiling, total RNA was isolated from the aqueous phase of lysed pgMECs and reverse transcribed into complementary DNA (cDNA), which was subjected to new generation sequencing (NGS) as described in Ref. [8]. Additionally, reverse transcription polymerase chain reactions (RT-PCR) were performed to monitor expression of the selected cell-type specific markers in the culture. Real-time quantitative polymerase chain reaction (RT-qPCR) method was used to determine relative quantities of markers and ratios between them (e.g. expression of caseins in basal and lactogenic medium). Due to poor annotation of the goat genome, PCR primers were designed against *Bos taurus* RefSeq (NCBI) mRNA sequences and cross-matched against the goat reference sequences, if available. We determined expression of markers on mRNA level first and proceeded to protein level (e.g. immunostainings and western blotting) afterward.

In order to detect beta-casein (CSN2) mRNA culprits, we performed reverse transcription polymerase chain reaction (RT-PCR) on pgMEC-derived transcriptome library (cDNA), using the following primer pair CSN2-F: 5′-ACAGCCTCCCACAAAACATC-3′, CSN2-R: 5′-AGGAAGGTGCAGCTTTTCAA-3′. The resulting 206 bp product was isolated from agarose gel, using gel extraction kit, and sequenced by Sanger sequencing to validate that the sequences correspond to the portion of the exon seven of the CSN2 gene (GenBank: AJ011019).

Real-time quantitative polymerase chain reactions (RT-qPCRs) consisted of 2× SYBR Green PCR master mix (Life Technologies), water, and 0.5 μM of each primer in a total volume of 20 μl. The cycles were as follows: 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Melting curve was determined at 15 s for 95°C, 1 min at 58°C, and 15 s at 95°C. The following primers were used: estrogen receptor 1 (ESR1) forward: 5′-ACAGCATGAAGTGCAAGAACGTGG-3′ and reverse: 5′-TGCAAGGAATGCGATGAAGTGCAG-3′; progesterone receptor (PGR) forward: 5′-AAGCCAAGCCCTAAGCCAGAGAAT-3′ and reverse: 5′-AGCTGGAGGTATCAGGTTTGCTGT-3′; and CSN2 forward: 5′-ACAGCCTCCCACAAAACATC-3′ and reverse: 5′-AGGAAGGTGCAGCTTTTCAA-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as an endogenous control, using the primer pair: forward: 5′-CATGTTTGTGATGGGCGTGAACCA-3′ and reverse: 5′-TAAGTCCCTCCACGATGCCAAAGT-3′.

2.2.2. Immunostainings

The immunostaining protocols often differ for different markers and every marker might require optimization of the protocol. Generally, the protocol consisted of cell fixation for

30 s to 1 min in ice cold acetone/methanol (1:1) or for several minutes in 4% paraformaldehyde. Fixation was followed by permeabilization (not necessary when using acetone-methanol fixation or in case of membrane-bound markers) with 0.3% Triton X-100 for 10 min. After washing with phosphate buffered saline (PBS), cells were blocked with 5–10% fetal serum (it is recommended to use fetal serum from species in which secondary antibodies were produced) and 1–3% bovine serum albumin (BSA) for 60 min. Incubation with primary antibodies was performed overnight at 4°C. Next day, cells were washed with PBS several times and incubated with fluorescently labeled secondary antibodies at room temperature for 1 h. After washing with PBS, cell nuclei can be counterstained with 4′,6-diamidino-2-phenylindole (DAPI), washed, and visualized under microscope. In case of paraffinized tissue, sections were deparaffinized using xylene and rehydrated in decreasing concentrations of ethanol. Rehydrated tissue slices were washed in PBS, followed by performing heat-induced antigen retrieval in a microwave oven, using 10 mM sodium-citrate buffer (pH 6). Afterward, the same protocol was used for immunofluorescent staining as described previously for pgMECs. The more detailed protocols and the antibodies used were described in our previous publications [8–10].

2.2.3. Mammosphere formation assay

When performing mammosphere formation assay, a single-cell suspension of the mammary cells was grown in DMEM/F12 medium, supplemented with EGF (20 ng/mL), bFGF (human, 20 ng/mL), heparin (4 μg/mL), cholera toxin (10 ng/mL), hydrocortisone (0.5 μg/mL), insulin (0.5 μg/mL), and B27 supplement (2%), and grown in 6-well ultralow-attachment plates with or without extracellular membrane matrix or in hanging drops, according to the described protocol [11].

2.2.4. Oil Red O staining

Growth medium was aspirated and the pgMECs were fixed in 4% paraformaldehyde for 15 min. Oil Red O (0.5 g) was dissolved in 50 ml of isopropanol and diluted with water (3:2) and then left for 10 min, and the solution filtered through a 20-μm filter. Cells were briefly washed with isopropanol (60%) and incubated with solution of Oil Red O for 15 min at room temperature. The cells were then rinsed with isopropanol and washed under tap water. The formation of lipid droplets (red stain) was observed under bright field microscope.

3. Mammary tissue–derived primary cells as an *in vitro* model

Mammary gland development occurs in stages; proliferation and differentiation of mammary cells are dependent on sexual development and reproduction, which are under control of endocrine system [12]. The gland is primarily composed of mesenchymal and epithelial tissue and the latter is subjected to significant remodeling during lactation cycles. The tissue remodeling involves proliferation and differentiation of the epithelial cells forming functional

glandular tissue, followed by regression of the tissue due to apoptosis and loss of glandular structure and function (involution). The functional part of mammary gland is glandular tissue connected by a branched system of secondary ducts, combining into larger primary ducts, which end in the gland cistern. The milk is synthesized by secretory luminal cells arranged in spherical structures called alveoli, which form larger structures called lobules. Alveoli and ducts of lactating mammary gland are composed of different types of epithelial cells important for milk synthesis and secretion, while connective and fat tissue surround and support the epithelial structures. An alveolus is comprised of a single layer of milk secreting luminal epithelium, surrounded by a single layer of contractile myoepithelial cells, which lie adjacent to the basal membrane, where mammary stem/progenitor cells also reside [10].

In agreement with the "Replacement" of the three Rs principle (3Rs: Replacement, Reduction, and Refinement) adequate *in vitro* model, mimicking the function of the mammary gland allows the study of physiological, biochemical, and immune functions of the mammary gland, substituting *in vivo* experiments. In addition to the ethical issues, cell lines enable use of many technical replicas, better control of the environment, and surmount the problem of variation introduced by animal's individuality [13] and the problem of systemic effects, which makes elucidation of a contribution of a particular cell type of interest difficult [14]. A main limitation of primary cell models is a finite life span, and a limiting number of available biological replicas, as a derived cell culture, represent a single genotype, while establishment and characterization of a large number of cell cultures/lines are quite a challenging and laborious process. Additionally, cell cultures do not always properly model *in vivo* conditions; therefore, limitations should be considered for each individual purpose.

Several ruminant immortalized mammary epithelial cell lines as MAC-T [15] and BME-UV [16] were generated by genomic integration of Simian virus large T-antigen (SV40LTA). However, transformed mammary cells are genetically and (usually) phenotypically changed. Transient mammary lines show low responsiveness to lactogenic hormones [17] and are not proper approximation of *in vivo* lactation. Genetic modifications and adaptations to growth in cell cultures alter metabolic pathways in continuous cell lines; therefore, the use of primary cells is a much better approximation of the *in vivo* system [18].

4. pgMEC characteristics

4.1. Morphology and growth

The derived primary cell culture consisted of a heterogeneous population of mostly epithelial and mesenchymal (fibroblast-like) cells. Epithelial cells grew in round-shaped densely packed islands of cells with multiple nucleoli and exhibited typical cobblestone morphology. Cells randomly spreading around these islands were larger, spindle-shaped cells, morphologically resembling fibroblasts (**Figure 2**).

Cell proliferation was slow for the first week after seeding dissociated cells in plastic dishes. After the first passage, the cells started to proliferate much faster and overgrew the surface

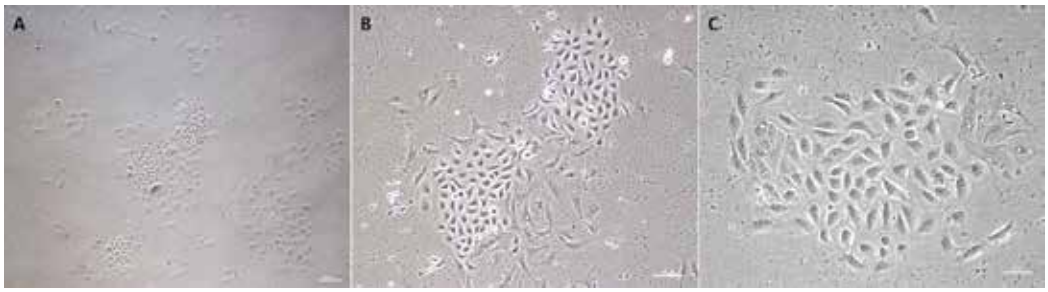

Figure 2. Primary culture 5 days after seeding under bright field microscope (photo: J. Ogorevc). (A) Heterogeneous cell types visible in the primary culture (40× magnification; scale bar = 200 μm). (B) Islands of epithelial cells surrounded by mesenchymal cells (fibroblasts). Cell debris can be observed in primary culture prior to first passaging (100× magnification; scale bar = 100 μm). (C) Densely packed island of epithelial cells (200× magnification; scale bar = 50 μm).

every several days. No changes in proliferation, morphology, or growth patterns were noticed for over five passages (**Figure 3**). When cells were kept at full confluency (without passaging) for extended period of time, they started to show signs of senescence.

4.2. Expression of specific markers

Different cell types express cell type–specific genes, which can be considered characterization markers. A draft of such markers was used to characterize the derived pgMECs and to distinguish different cell types in a heterogeneous primary cell culture.

Transcription profile generated by NGS showed that markers typical of basal/myoepithelial and luminal epithelial cells were highly expressed. The most expressed were different keratins, desmoplakin, and actins. The expression of markers varies based on the number of different cell types/lineages present in the culture and culture conditions, which may favor proliferation of a specific cell type and may promote differentiation (e.g. epithelial-to-mesenchymal transitions).

Figure 3. Mammary cell lines after passaging (photo: J. Ogorevc). (A) Epithelial and mesenchymal cells under 40× magnification (scale bar = 200 μm). (B) Island of epithelial cells (right) and mesenchymal cells (left) (200× magnification; scale bar = 50 μm). (C) Enriched culture of epithelial cells after differential trypsinization and removal of fibroblasts (200× magnification; scale bar = 100 μm).

The method of choice for characterization of different cell types in a cell culture is staining cells with tissue/cell type–specific antibodies, which reveal presence and localization of markers in the cells. The analysis of whole-transcriptome mRNA expression and review of previous studies, regarding distinctive mammary-specific markers in different species, represented a rationale for selection of antibodies, potentially useful for characterization of major cell types in goat mammary tissue and the derived cell cultures. Antibody-based characterization is a challenge in ruminants (especially goats) as most of the commercially available antibodies are targeted against human or rodent antigens, while their reactivity in ruminants is generally unknown and has to be determined empirically. To determine the presence of mammary-specific protein markers, immunofluorescent staining with different antibodies was performed.

Based on our results, we suggest cytokeratins (KRT) 14 and 18, as well as vimentin (VIM) as suitable markers for basic characterization of primary mammary cell cultures (**Figure 4**). Namely, cells of mesenchymal origin (e.g. fibroblasts) express VIM (**Figure 4A** and **B**), KRT 14 is distinctive of myoepithelial (**Figure 4E** and **F**), whereas KRT18 of luminal epithelial cells (**Figure 4C** and **D**). Based on these three markers, it is possible to distinguish epithelial cells from mesenchymal cells and distinguish between basal/myoepithelial and luminal cells.

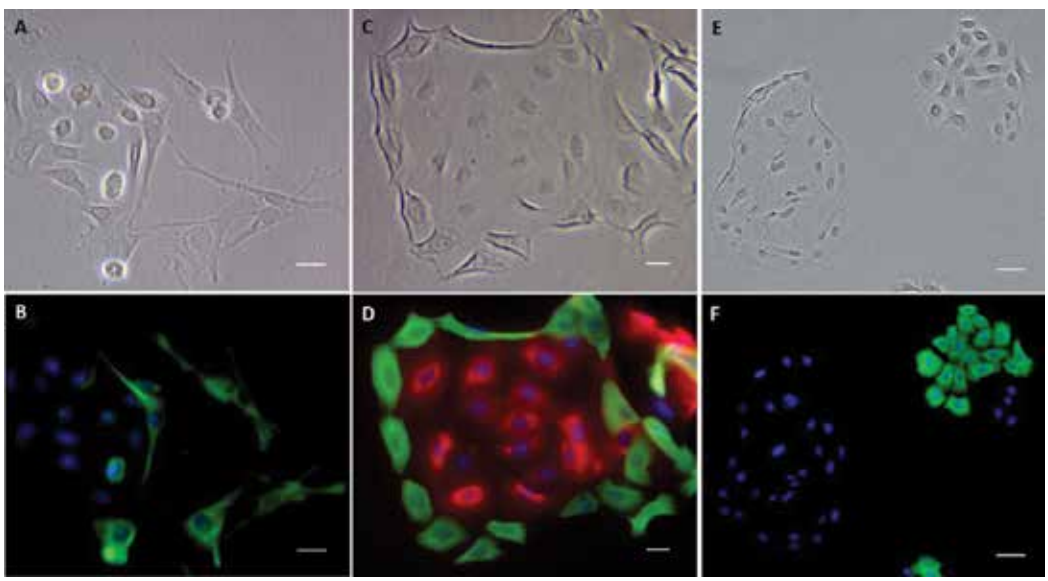

Figure 4. Basic characterization of the pgMECs. Fixed pgMECs under bright field (A, C, and E) and fluorescent illumination (B, D, and F) under 40× magnification (A–D; scale bars = 20 μm) and 20× magnification (E and F; scale bars = 50 μm) (photo: J. Ogorevc). Fluorescently labeled secondary antibodies were used to visualize expression and localization of the markers and a DAPI counterstain was used to visualize the nuclei. (A and B) The cells immunostained with primary antibodies against VIM. Spindle-shaped fibroblasts stained for VIM. (C and D) Double staining for KRT14 and KRT18. Luminal epithelial cells stained for KRT18 (D), whereas myoepithelial for KRT14 (D). Interestingly, when grown at low confluency, cells tended to organize as in alveoli, myoepithelial cells encircling luminal cells. (E and F) Staining for KRT14. Two islands of epithelial cells visible; myoepithelial cells stained for KRT14 (upper right corner), whereas no staining with KRT14 is visible in luminal epithelial cells (left).

Additional markers useful to distinguish epithelial cells from other cell types and to determine epithelial subtypes are different keratins (e.g. 5, 19), epithelial cell adhesion molecule (EPCAM), estrogen receptor 1 (ESR1), tumor protein p63 (TP63), integrin subunit beta 1 (ITGB1/CD29), integrin subunit alpha 6 (ITGA6/CD49f), progesterone receptor (PGR), alpha smooth muscle actin (ACTA2), caseins (e.g. CSN2), and mucin 1 (MUC1).

Additionally, paraffin-embedded sections of goat mammary tissue were stained to compare the expression of the markers between the pgMECs and the mammary tissue (**Figure 5**). Most of the markers showed reactivity in both—the cell cultures and the tissue, whereas some of the markers showed reactivity only in pgMECs (ESR1, CD49F, and KRT5) or only in the tissue (TP63). Tissue sections undergo chemical and physical treatment, which might result in changed conformation and antigen masking. On the other hand, pgMECs adapt to *in vitro* environment, which may alter cell metabolism and expression of markers. Therefore, discrepancies in immunostaining results are possible between the tissue and pgMECs. For example, EpCAM marker was localized in cytoplasmic compartment of pgMECs (**Figure 5A** and **B**) and was also found in epithelial compartments of goat mammary tissue (**Figure 5C** and **D**). In case of MUC1, weak signal was observed in pgMECs (**Figure 5E** and **F**) and strong signal, showing distinctive localization of MUC1 only to apical plasma membranes of secretory (luminal) epithelial cells, was detected in the mammary tissue (**Figure 5G** and **H**).

4.3. 3D organization—mammosphere formation

Under conditions that do not allow adherence to the surface, differentiated epithelial cells undergo anoikis. Growth under nonadherent, serum-free conditions is a characteristic of mammary stem/progenitor cells, which in such conditions form spherical structures called mammospheres. Spherical structures formed by human mammary epithelial cells contain enriched population of cells capable to differentiate into luminal or myoepithelial cells (bipotent progenitors) [19]. The molecular and cellular processes in mammospheres are similar as those in developing alveoli of the mammary gland [20]. Hierarchically, mammary cells range from terminally differentiated cells to undifferentiated progenitors and stem cells, the latter two being likely targets for malignant transformations in cancer [21]. It was shown that an entire mammary gland can be reconstituted from a single mammary stem cell [22]. Existence of mammary stem/progenitor cells in goat was first demonstrated by [10].

Under nonadherent conditions, irregularly shaped floating masses (organoids) were formed after several days. Aggregates that arose in ultralow-attachment plates (**Figure 6A**) in medium supplemented with basement membrane matrix were rounder and larger in shape as those grown in medium without basement membrane matrix. Immunostaining of fixed mammospheres revealed that luminal (KRT18—positive) and basal/myoepithelial (KRT14—positive) cells were the main constituents of the mammospheres [8]. Additionally, mammospheres were grown using hanging drop method. Hanging drop method is used as one of the *in vitro* tests for determining the pluripotent character of putative stem cells. The spherical structures appeared after several days of growth in hanging drops. They were fewer in number, but larger and more round in shape (**Figure 6B**), compared to mammospheres grown in ultralow-attachment plates. The mammospheres were fixed to glass slides and stained with DAPI and

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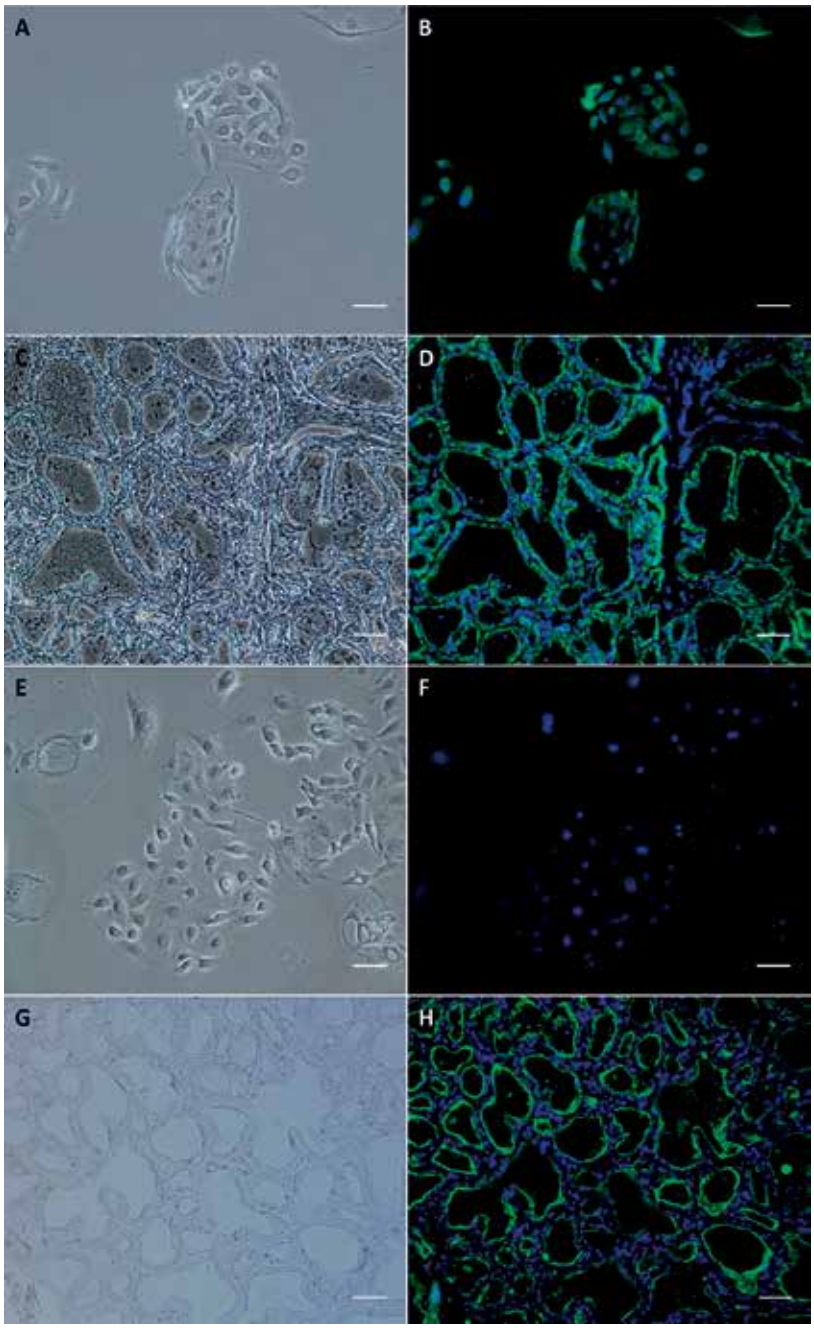

Figure 5. Immunofluorescence of pgMECs and goat mammary tissue under bright field and fluorescent illumination, stained against EpCAM (A–D) and MUC1 (E–H) (20× magnification; scale bars = 50 μm) (photo: J. Ogorevc). Fluorescently labeled secondary antibodies (green) were used and a DAPI counterstain was used to visualize nuclei (blue). The mammary cell culture (A and B) and epithelial cells of the tissue (C and D) stained against epithelial cell–specific marker EpCAM. pgMECs showed weak staining against MUC1 (E and F), whereas strong signal, localized to apical membranes of alveolar structures, was observed in the tissue (G and H).

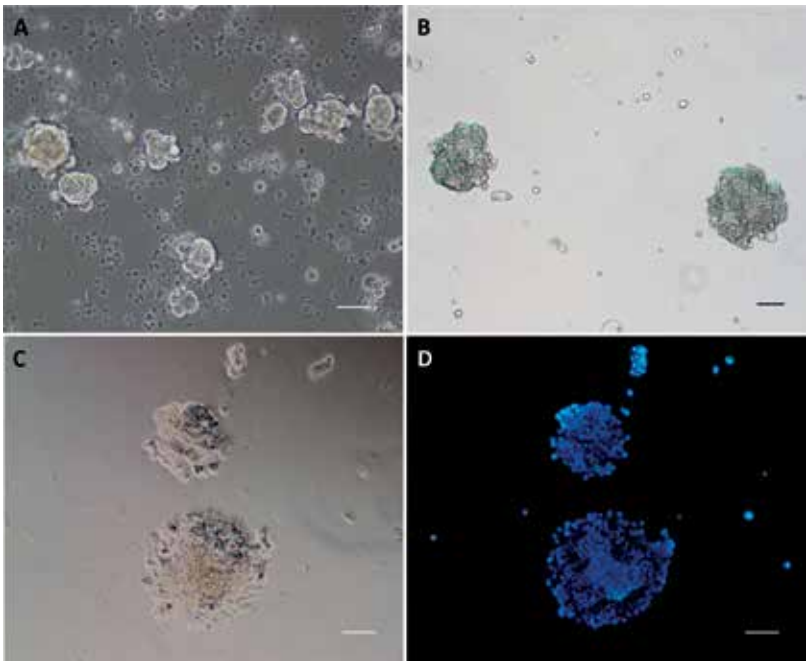

Figure 6. Formation of spherical structures in the pgMECs after 7 days of growth under nonadherent conditions (photo: J. Ogorevc). (A) Spherical structures in low-attachment plates (20× magnification; scale bar = 50 μm). (B) Organoids (mammospheres) grown in hanging drops (4× magnification; scale bar = 200 μm). (C and D) DAPI-stained fixed mammospheres, grown in hanging drops under bright field (C) and fluorescent illumination (D) (100× magnification; scale bar = 100 μm). Number of cells in the organoid structures can be estimated based on the number of stained nuclei (blue).

antibodies raised against KRT14 and 18. They consisted of several hundred cells (**Figure 6D**), which were KRT14- or KRT18-positive. Hanging drop is an efficient method to grow mammospheres from primary mammary cultures. The cells avoiding anoikis and forming organoids probably represent mammary progenitors.

5. Practical applications of the pgMECs

Mammary development, hormone responsiveness of mammary cells, regulation of milk expression, modeling milk composition and coagulation properties, enhancing milk yield, and innate immunity are some of the interests of modern dairy production. The characterized mammary cell lines are useful models to study biology of the mammary gland. For example, we used the cells for infection study with a common mastitis-causing agent in goats—*Mycoplasma agalactiae* (PG2 strain)—and to study differences in expression of the steroid receptors and beta casein in different growth conditions and in different pgMEC lines, derived from tissues of animals in different physiological states.

5.1. Mastitis model

Because of the economic importance for dairy industry and possible health and milk quality risks for consumers, there is a great interest to understand and enhance natural immunity of the mammary gland. Mammary epithelial cells are capable of innate immune response during intramammary infections and represent important barrier against invading pathogens.

In small ruminants, coagulase-negative staphylococci account for most of the mastitis cases, followed by Streptococci, *Staphylococcus aureus*, and other bacteria [23]. Additionally, contagious agalactia caused by *Mycoplasma agalactiae* (*Ma*) is a common cause of intramammary infections (contagious agalactia), especially in Mediterranean regions [24]. Mammary cell lines are often used to study immune response to mastitis, instead of *in vivo* infections. In our study, next-generation sequencing (NGS) was used to assess whole-transcriptomic response of *Ma*-challenged pgMECs 3, 12, and 24 h postinfection [25].

The results show that the infection induced an innate immune response in the infected cells. The pgMECs were capable of recognizing and responding to the pathogen infection (**Figure 7**). The pgMECs responded by induced expression of cytokines (interleukins and chemokines) and other chemotactic agents, activation of complement system, apoptosis pathways, and induction of genes coding for antimicrobial effector molecules (e.g. defensins, lysozyme, and nitric oxide synthase) (**Figure 7A**). The changes in expression were moderate, with no phenotypically visible changes in cell morphology, which corresponds to subclinical course of contagious agalactia *in vivo*. The pathway enrichment analysis showed that the most affected pathways were associated with immune response, steroid and fatty acid metabolism, apoptosis signaling, transcription regulation, and cell cycle regulation. We speculate that physiologically, the *in vivo* immune contribution of the pgMECs is important for recruitment of

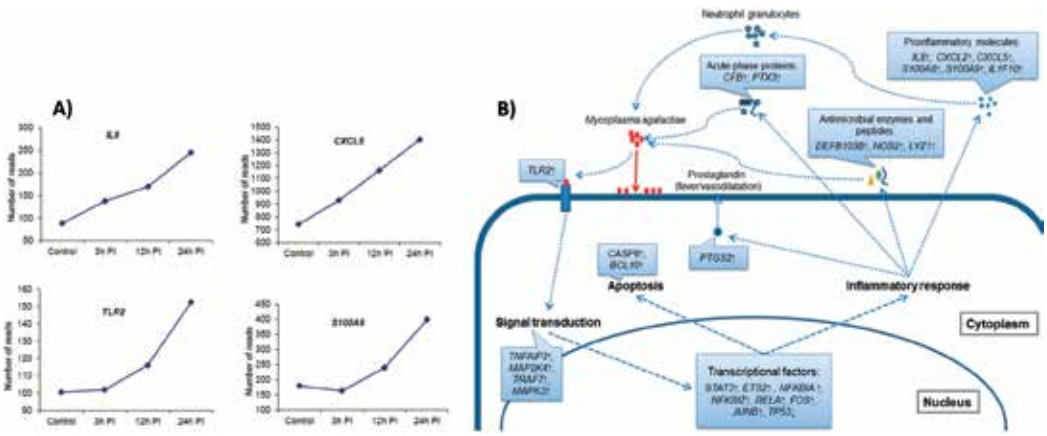

Figure 7. Transcriptomic studies on *Mycoplasma agalactiae*–infected pgMECs (modified from Ref. [25]). (A) Induction of immune-associated genes interleukin 8 (IL8), chemokine (C-X-C motif) ligand 5 (CXCL5), Toll-like receptor 2 (TLR2), and S100 calcium-binding protein 9 (S100A9). (B) Possible immune response mechanisms in pgMEC, suggested based on differential expression of genes and analysis of genetic networks and metabolic pathways.

neutrophils, activation of complement system and apoptotic pathways, as well as expression of several bactericidal molecules (**Figure 7B**) [25].

5.2. Mammary differentiation and lactation model

Expression of milk proteins and appearance of milk drops containing lipids are signs of lactogenic differentiation of the mammary cell culture. Lactogenic differentiation is dependent on multiple factors. To determine if the derived pgMECs are capable of lactogenic differentiation, we performed screening for beta-casein expression, the most abundant protein in goat milk, and stained putative milk drops for the presence of lipids, using Oil Red O.

Several pgMEC lines from mammary tissue of animals in different physiological states (different stages of lactation and juvenile goats) were established and grown under different growth conditions (basic and lactating). We observed different morphology, expression of steroid receptors (estrogen and progesterone), and expression of beta casein (CSN2), and tried to evaluate the effect of different growth conditions (medium, growth surface, and cell density) and donor tissue on characteristics of the derived cell lines. It was shown that primary mammary cells rapidly loose expression of steroid receptors [26] and casein [27] when grown in monolayer on plastic, whereas growth on extracellular matrices should provide the basal-apical polarization to the epithelial cells, needed for maintaining certain characteristics or to enable proper differentiation and milk component synthesis [28, 29].

5.2.1. Morphology

Morphology differed between the derived primary cultures established from mammary tissue of goats in different physiological states. Cells of the same primary cell line, grown under different growth conditions, also exhibited morphological differences (**Figure 8**). When cells were grown to confluency in lactogenic medium, dome-like and acini-like structures were formed in pgMECs, derived from the lactating tissue. No such structures appeared in pgMECs derived from the tissue of juvenile goats, grown under the same conditions. However, lumen-like and milk drop–like structures were formed only in pgMECs derived from juvenile goats, grown in lactogenic medium.

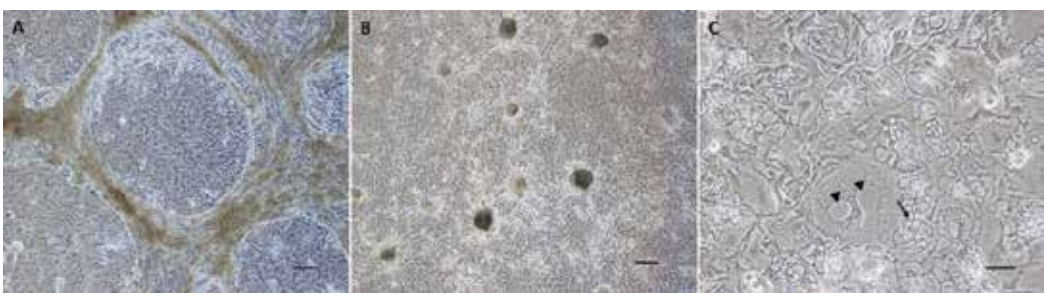

Figure 8. Morphology of different pgMEC lines in lactogenic conditions (photo: J. Ogorevc). Dome-like (A) and acini-like structures (B) were formed in pgMECs, derived from lactating mammary tissue (40× magnification; scale bars = 200 μm). Vacuoles resembling milk drops (arrow) and lumen-like structures (arrowheads) were formed in pgMECs, derived from juvenile goats (200× magnification; scale bar = 50 μm).

5.2.2. Expression of the steroid receptors

Terminal differentiation of mammary epithelial cells is required for luminal cells to secrete milk. Estrogen and progesterone are important hormones in mammary development and mammary cell differentiation. The roles of estrogen and progesterone during morphogenesis are well known [12], but their role during lactation is not clear. The function of the hormones is mediated through estrogen (ESR) and progesterone receptors (PR) that in ligated form migrate to nucleus and act as transcription factors for various genes. The studies suggest that ESR1 and PR are (co)expressed in mammary epithelial cells [30] where they participate in regulation of differentiation and control balance between luminal and basal mammary epithelial cells, mediated through paracrine signaling between the neighboring cells [31, 32]. Most of the ESR1- and PR-positive cells express markers of the luminal lineage [33], whereas the lack of ESR and PR is typical for undifferentiated mammary progenitor cells [34]. Unclear mechanisms by which hormonal action regulates lineage commitment and cell proliferation hamper our understanding of mammary differentiation, potentially useful for boosting milk production, as well as for better understanding of malignant transformations in mammary cells.

We quantified the expression of the steroid receptors in different pgMEC lines, grown in different conditions [35]. The cell lines derived from the mammary tissue of nonlactating doelings exhibited higher relative expression of ESR1 (approximately 50-fold) and PGR (approximately 8-fold), compared to cells derived from tissue of lactating goats. The response to lactogenic conditions was variable upregulation (from 1.4- to 12-fold) of ESR1 and consistent (approximately 3-fold) downregulation of PGR. Using immunostainings, we identified epithelial cells negative for both receptors, positive solely for ER-α or PR, and cells coexpressing both receptors. ER-α and PR were mainly localized in the nuclei and partly in cytoplasm of the cells. Multiple staining with luminal (CK18) or basal (CK14) markers revealed that not all of the ER-α–positive or PR-positive markers belonged to the luminal lineage and that not all of the luminal cells are ER-α and/or PR-positive (**Figure 9**). It seems that lactogenic conditions caused differentiation and proliferation of the luminal lineage and that ER-α could be involved in functional differentiation of the luminal mammary cells.

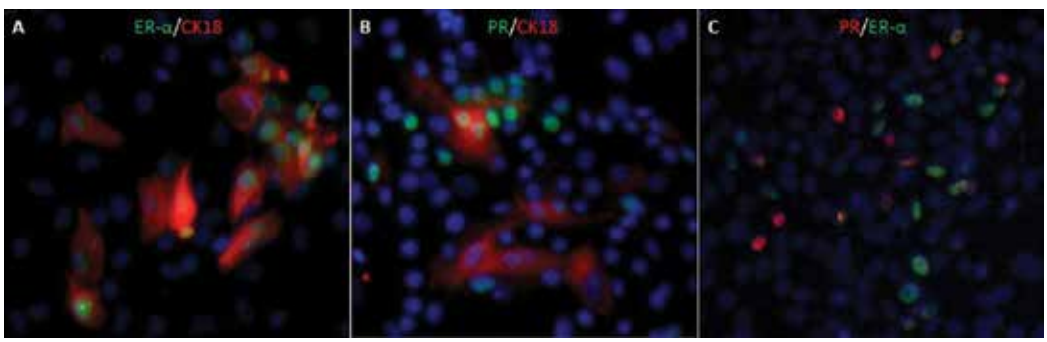

Figure 9. Immunofluorescent double stainings of the pgMEC lines, under 200× magnification (photo: J. Ogorevc). Nuclei were counterstained with DAPI (blue). (A) ER-α (green) localized mainly in nuclei of CK18-positive (red) cells. CK18-negative cells with nuclear staining against ER-α can also be observed. (B) PR-positive (green) and PR-negative luminal (red—CK18-positive) cells. (C) Nuclear colocalization of ER-α and PR (orange) and cells positive solely for ER-α (green) or PR (red).

5.2.3. Expression of beta casein (CSN2)

Expression of caseins was detected in various primary and immortalized mammary cell lines from different species. For example, mouse HC11 cells [36], several bovine mammary cell lines [15, 28, 37–39], and goat primary mammary cells [8, 18, 40] are able to express caseins. Transcription of CSN2 was studied in mouse HC11 cells and the authors [41] found that its expression is induced synergistically by combination of lactogenic hormones, local growth factors, and cell-cell and cell-substratum interactions.

We evaluated how the starting tissue material, addition of hormones (insulin, hydrocortisone, and prolactin) to the growth medium, and growth on a commercially prepared extracellular basement membrane matrix affect relative expression of beta casein [42], determined using RT-qPCR (**Figure 10A**). The CSN2 transcripts were detected in all of the samples, including cells originating from nonlactating goat, grown in basal medium. However, the expression of CSN2 and response to different growth conditions were different in individual cell lines. Interestingly, we found that CSN2 expression was the highest in pgMECs derived from juvenile goats, grown in lactogenic medium. Addition of hormones in most cases induced expression of CSN2. The effect of extracellular membrane matrix–covered growth surface (Geltrex in our case) was variable. We found that extracellular membrane matrix growth surface was not indispensable for casein expression in the cell lines. In some of the cell lines, membrane matrix significantly increased CSN2 expression, whereas in others, it did not have a statistically significant effect (in several cases, expression of CSN2 was even lower [nonsignificantly] in pgMECs grown on membrane matrix). We speculate that some of the cell types present in a heterogeneous mixture of the cell cultures, are capable of extracellular matrix production in quantities sufficient for luminal cells to achieve lactation competency. It was reported previously that mouse HC11 cells can produce extracellular matrix [43] and do not require additional matrix for CSN2 expression [36].

Complex regulatory mechanisms are required for the onset of lactation, which involve functional differentiation/proliferation of the mammary cells and considerable anatomical and physiological tissue perturbations during gestation. Interestingly, milk proteins can be detected in pgMECs already after several days of growth in cell culture, even if derived from the tissue of nonlactating animals. It seems that pgMECs are capable of terminal differentiation from basal to secretory cells in a short period of time, when grown in environment enabling lactogenic differentiation. CSN2 was localized mainly in circular (lumen-like) structures, formed only by pgMECs grown in lactogenic conditions (**Figure 10B**). To conclude, expression of CSN2 in pgMECs is variable and depends mostly on starting tissue material and growth conditions.

5.2.4. Lactating versus juvenile mammary tissue–derived pgMECs

The expression of the steroid receptors and beta casein was the highest in juvenile mammary tissue–derived pgMECs, which could indicate a possible role of ER and PR in lactogenic

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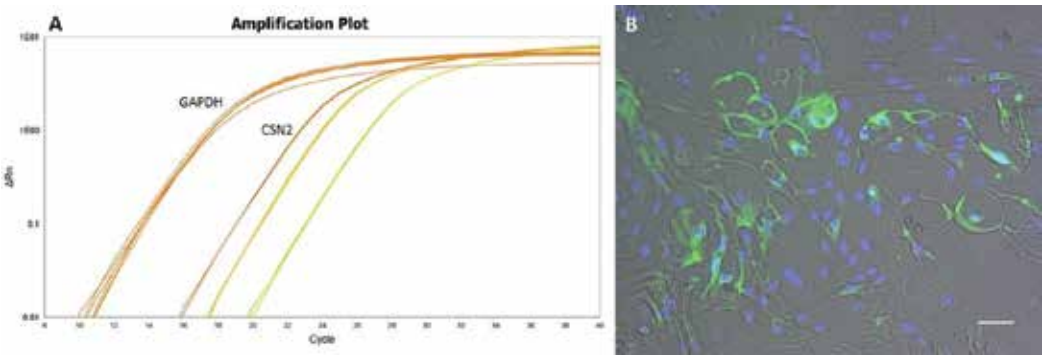

Figure 10. Expression of CSN2 mRNA and localization of the protein in pgMECs (photo: J. Ogorevc). (A) RT-qPCR amplification plot for housekeeping GAPDH and CSN2 in three different pgMEC lines. (B) Beta casein was present in pgMEC lines and localized mainly in vacuoles (lumen-like structures), formed only under lactation-inducing conditions (magnification 200×; scale bar = 50 μm).

differentiation and proliferation of mammary epithelial cells. Research on humans shows that ER-/PR-positive mammary cells represent early mammary progenies and regulate differentiation of ER-negative mammary progenitors, from which basal/myoepithelial cells arose, and to ER-positive bipotent progenitors, which can give rise to luminal and myoepithelial lineage [33, 44]. It would make sense that progenitor fractions are enriched in juvenile mammary tissue (and in derived pgMECs) where lactogenic differentiation had not occurred yet. Prpar and colleagues [10] reported existence of different mammary progenitors in goats, luminal-restricted, myoepithelial-restricted, and bipotent and showed that the tissue from animals at the peak of lactation and from a juvenile animal contained the highest number of luminal progenitors, whereas the tissue at the onset of involution contained mostly myoepithelial progenitors. In our experience, tissue from young, nonlactating (juvenile) goats seems more suitable for preparation of lactation-competent cell cultures than tissue from lactating or involuting animals. Similar was also suggested in case of bovine primary mammary cells and attributed to a better proliferation capability of such cells [38].

6. Conclusions

The development of primary cell lines from lactating, juvenile, and involuting goat mammary tissue has been described. The derived pgMECs were maintained in cell culture for several passages without signs of further differentiation or senescence. The extensive characterization of established cell lines was performed and the main cell types in the mammary culture were determined. The pgMECs were capable of innate immune response and remained hormone responsive. Under lactogenic conditions, the cells successfully change morphology, synthesize milk proteins, and form lumen-like and milk drop–like structures. The established cell lines represent an adequate model of goat mammary tissue, useful for basic and applied research in mammary gland biology and biotechnology.

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Section 5

Milk and Health

Chapter 10

Nutritional and Health Profile of Goat Products: Focus on Health Benefits of Goat Milk

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Additional information is available at the end of the chapter

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Abstract

Goat (*Capra hircus*) is one of the main sources of milk and meat products for human consumption. Goat milk differs from cow and human milk in both composition and nutritional properties. Goat milk and other goat-derived products contain several bioactive compounds that might be useful in patients suffering from a variety of chronic diseases. Several peptides, fats, and oligosaccharides present in goat's milk can be potentially useful in cardiovascular disease, metabolic disorders, neurological degeneration, or in promoting intestinal health. They have also shown chemopreventive properties in cancer. In addition, the oligosaccharides present in goat's milk have immunomodulatory properties, prevent adhesion of pathogenic bacteria, and have prebiotic, probifidogenic effects. Due to its potential health benefits, goat milk is particularly recommended for infants, older adults, and convalescing people. This chapter gives an overview of the biological activities of goat products and the effects of peptides, fats, and oligosaccharides present in goat milk on pathogenic bacteria, as well as their ability to regulate immunological, gastrointestinal, hormonal, and neurological responses in humans.

Keywords: goat milk composition, goat's products, nutritional value, bioactive compounds, health effects

1. Introduction: the importance of goat milk, dairy products, and meat as a potential functional food

Interest from the consumers as well as growing concerns for physical well-being and health have increased the demand for information about the consumption of healthier foods.

The intake of some active compounds present in food, the so-called nutraceuticals and functional foods, can be regarded as having beneficial effects (physical and mental) on certain functions in the human body, that go beyond their nutritional effects.

As technology and science advances, the fields of health and nutrition have focused on several emerging fields, namely nutrigenomics, or "personalized nutrition." The study of the human genome in order to comprehend cellular response to nutrients and bioactive compounds is a promising field of work, which should lead specific dietary recommendations to prevent or aid in the treatment of certain diseases. In fact, stroke, cancer, and atherosclerosis and the general risk of diseases may be somehow minimized by introducing proper preventive nutrition and functional foods/foods as part of a healthier lifestyle that includes a balanced diet and physical activity [1]. Ingesting a wide variety of foods, namely fruits, vegetables, whole grains, milk, meat, and eggs is one way to ensure the intake of certain bioactive compounds that are present in its constitution such as carotenoids, dietary fiber, fatty acids, flavonoids, isothiocyanates, minerals, phenolic acids, plant stanols/sterols, polyols, prebiotics and probiotics, phytoestrogens, soy protein, sulphides/thiols, and vitamins [2].

Functional foods are growing in reputation across the globe and are becoming a part of daily diet of consumers who are concerned with their health. The global market potential for functional foods and beverages has been estimated to be worth $192 billion by 2020 [3]. However, the effectiveness of nutraceutical products in preventing diseases depends on preserving the stability, bioactivity, and bioavailability of the active ingredients [4]. Functional foods are found virtually in all food categories; however, some products are not widely available in the market [5].

Sheep and goat products (mainly meat and dairy) have interesting characteristics in their levels of flavor, taste, aromas, and leanness as well as the specific composition of fats, proteins, amino, and fatty acids and have been traditionally consumed in certain regions of the globe [6]. Additionally, the nutritional properties of goat milk and its lower allergenicity when compared to cow milk [7, 8] has sparked an interest in goat milk as a functional food, and it is now one of the current trends in healthy eating in developed countries [9]. Moreover, the use of milk with particular nutritional properties, alone or in combination with bacterial strains with probiotic properties and capable of producing physiologically active metabolites, might become one of the options for manufacturing new dairy functional beverages [10].

2. Materials and methods

The present chapter intends to give a comprehensive approach of the unique characteristics of goat-derived products, which have attracted the interest of researchers worldwide. This chapter aims to explore the nutritional value and bioactivity of the constituents of these products, with an emphasis in the reduction of the risk of chronic disorders by anti-inflammatory and anti-oxidative effects. The ability of goat's milk and its derivatives to selectively encourage bacterial growth in intestinal microbiota and the beneficial effects in the metabolic, endocrine, and immune systems will also be a subject of this chapter.

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3. Composition of goat milk

Milk and derived dairy products are considered an important constituent of a balanced diet. Milk, as the first food for mammals, supplies all the energy and nutrients needed for the proper growth and development of the neonate. For all mammalians, the consumption of milk ends at the weaning period except in humans, which continue consuming milk throughout their life.

The physical characteristics and composition of milk vary between species. Chemically, milk is a complex oil-in-water emulsion containing proteins, fats, carbohydrates (mainly lactose), and lower amounts of minerals, enzymes, cells, hormones, immunoglobulins, and vitamins.

The information currently available on the composition of goat milk has been published in the form of reviews [11–13]. Authors are unanimous in recognizing that fresh milk composition has a dynamic nature that varies with several factors such as (a) genetics (e.g., species, breed, and individual); (b) stage of lactation; (c) health status of the individual animal; and (d) environmental factors (e.g., feed, climate, season, or method of milking) [14–16].

Approximate compositions of the milks of different animals are compared in **Table 1**.

The basic nutrient composition of goat milk resembles that of cow milk (**Table 1**). Both milks contain substantially higher amounts of proteins and minerals, but lower lactose content than human milk [17]. Nonetheless, goat milk has high concentrations of fat globules, which are smaller than those present in cow milk; these globule diameters average are approximately 3.6 and 3.0 μm against 4.0 μm, respectively [18, 19]. The smaller size of fat globules provides a smoother texture in goat's derived products. Furthermore, goat milk contains lower amounts of α1-casein conferring it a higher water-holding capacity and a lower viscosity [20, 21]. Despite all these properties, the flavor of goat's milk is peculiar and more intense in comparison to cow's milk, which can restrict the acceptance of its derivatives by consumers [21]. However, goat milk is more easily digested than cow milk due to the absence of agglutinins in the former [11].

Goat and cow milk differ essentially in their casein micelles (structure, composition, and size), the proportion of individual protein fractions and higher content of nonprotein nitrogen and mineral compounds found in goat milk.

Composition	Goat	Cow	Human
Energy (kcal)	70	69	68
Water (%)	87.5	87.7	86.7
Total solids (g)	12.2	12.3	12.3
Protein (%)	3.2	3.3	1.3
Fat (%)	4.0–4.5	3.8	4.1
Lactose (%)	4.6	4.7	7.2
Ash (g)	0.8	0.7	0.2

Table 1. Basic composition of different milks (mean values per 100g) (adapted from Yadav et al. [24]).

3.1. Proteins

The variability in milk composition, among individual animals of the same breed, is attributed to an extensive and complex genetic polymorphism of the goat milk caseins. The protein portion has a fundamental role on the nutritional and technological value of milk. Milk proteins are made of heterogeneous groups in terms of composition and properties and are divided into casein—the main group of proteins—and whey protein fractions to a lesser extent.

The total protein content in goat milk varies from 2.6 to 4.1 g/l. Casein is composed of four fractions: αS1-casein, αS2-casein, β-casein, and κ-casein [22]. The proportions of the milk casein fractions differ between ruminant species and the micelle characteristics also differ in regard to size, hydration, and mineralization. As reported by Grosclaude and Martin [23], in studies conducted using French Alpine and Saanen goats, milk from animals with low alleles FF had total casein and total protein contents of 22 and 27 g/l, respectively. These values increase to 27 and 32 g/l in milk from animals possessing strong alleles AA. Generally, goat milk contains less αS1-casein than other ruminants' milk. However, as allele frequency for this specific casein varies between breeds, its concentration in goat milk depends indirectly on the breed [23].

Other characteristics of goat milk proteins are their structural conformations and the amounts and subtypes of micelles, which are smaller (180 nm) than those of cow milk (260 nm) and similar to those of sheep milk (193 nm) [11].

It has been reported that beta casein comprises the largest fraction of total goat milk casein. Although αS2-casein is relatively higher in goat milk, the αS1 fraction of cow milk alone is higher than both the αS1 and αS2-casein fractions present in goat milk. These differences might help explain the soft curd-forming properties of goat milk, as well as its better digestibility and the lower frequency of allergic reactions in children [24].

The nutritional value of proteins present in milk depends on its essential amino acid content. Only small differences exist in milk amino acid levels per 100 g of protein between different species, which are most likely due to differences in total protein content [15, 25]. A comparison of amino acid content between different species can be seen in **Table 2**.

When compared to cow milk, goat milk has higher levels of essential amino acids: threonine, leucine, lysine, cystine, tyrosine, phenylalanine, valine, and nonessential proline and glutamic acid (**Table 2**) [26].

The importance of amino acid composition and polypeptides will be examined later in this chapter.

3.2. Fat

Fat content is the more quantitatively and qualitatively variable component of milk, depending on season, lactation stage, breed, genotype, and feeding. This last factor has been extensively studied, and Sanz Sampelayo et al. [27] examined the influence of feeding, roughage, and lipid supplementation on the fat content of both ewe and goat milk, and the presence of fatty acids of nutritional interest, such as rumenic acid or omega-3, as well as other fatty acids with potentially detrimental effects, such as trans fats. Chilliard et al. [28, 29] showed that goat's response

	Goat milk	Cow milk
Essential amino acids		
Thr	138.67	115.81
Ileu	160.54	128.04
Leu	341.01	266.23
Lys	342.86	252.59
Met	77.95	71.15
Cys	30.62	23.20
Phe	175.45	133.51
Tyr	162.51	159.99
Val	210.23	147.34
Total	**1639.84**	**1298.36**
Nonessential amino acids		
Arg	135.65	114.44
Hist	122.73	93.06
Asp	117.95	96.0.859
Ala	250.15	214.22
Glu	694.58	554.30
Gly	55.83	49.24
Pro	310.61	253.38
Ser	152.65	147
Total	**1840.15**	**1522.58**

Table 2. Amino acid composition of goat milk and cow milk (mg/100g milk) (adapted from Ceballos et al. [26]).

to lipid supplementation increased the fat content of goat milk without decrease in its protein content. Bovine, sheep, goat, and human milk fat consist of 97–98% of triglycerides, but have only low levels of phospholipids (0.5–1.5%) and free fatty acids (0.7–1.5%) [11].

The main characteristic of goat milk fat is the high content in short- and medium-chain fatty acids (MCFA). Average goat milk fat profile of fatty acids presented levels of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), and linoleic acids (C18:2) higher than those exhibited by cow milk. In contrast, goat's milk fat presented lower concentrations of stearic (C18:0) and oleic acid (C18:1) when compared to cow's milk fat. Average fatty acid composition (g/100g milk) of goat and cow milks are presented in **Table 3** [12]. Considering that these fatty acids have a different metabolism from that of long-chain fatty acids they present different functional proprieties [30]. Recent works of Núñez-Sánchez et al. [31] demonstrated that goat's milk exceeds cow's milk in its content of monounsaturated and polyunsaturated fatty acids and medium chain triglycerides. We will return to this subject later in this chapter.

Lipid profile	Goat milk	Cow milk
C4:0	66.55	116.44
C6:0	171.68	77.86
C8:0	192.20	57.80
C10:0	579.10	114.91
C11:0	7.46	7.29
C12:0	232.61	130.87
C14:0	518.56	384.41
C14:1	7.19	16.87
C15:0	28.11	35.23
C15:1	3.01	2.74
C16:0	1340.97	1102.72
C16:1	51.58	52.32
C16:2 n-4	1.57	0.57
C17:0	18.44	6.27
C17:1	4.32	2.85
C18:0	493.56	378.25
C18:1 n-9, trans	19.22	55.75
C18:1n-9, cis	1245.92	742.71
C18:2 n-6	142.39	82.31
CLA n-7, cis-9, trans-11	18.70	13.79
CLA n-6, trans-10, cis-12	3.53	1.82
CLA n-7, cis-9, cis-11	1.05	–
CLA n-5, cis-11, trans-13	12.42	–
CLA total	35.75	15.62
C18:3 n-3	27.72	8.55
C20:0	2.49	3.76
C20:1 n-9	1.57	1.03
C20:2 n-6	5.49	1.48
C20:3 n-6	-	0.80
C21:0	1.44	0.23
C22:0	4.05	3.99
C23:0	0.26	0.91
C24:0	0.66	0.68
C24:1 n-9	0.92	–
C6-14	1695.70	790.02
SFA	3683.10	2436.41

Lipid profile	Goat milk	Cow milk
MUFA	1342.67	874.27
PUFA	213.25	109.32
PUFA n-6	146.97	86.41
PUFA n-3	26.81	8.55
PUFA n-6/n-3	5.49	10.49

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 3. Fatty acid composition (mg/100g milk) of goat and cow milk fat (adapted from Ceballos et al. [26]).

3.3. Carbohydrates

Lactose is the main carbohydrate in milk: about 44% in goat milk and 49% in sheep milk. Its concentration does not vary excessively [32, 33]. However, goat milk lactose content is often largely increased by dietary plant oil supplementation in contrast to cow milk [34].

3.4. Minerals

Raynal-Ljutovac et al. [13] presented an update of the composition of goat and sheep milk products. In this document, authors complicated data available concerning the main mineral composition of goat and sheep milks (**Table 4**). Goat milk is distinguished by its high chloride and potassium content. A more recent study by Trancoso et al. [35], focusing on goat milk from the main Portuguese indigenous breeds (Serrana, Serpentina, Charnequeira, and Algarvia), also obtained similar results regarding mineral composition in the milk produced by these animals. Raynal-Ljutovac et al. [13] indicated that caprine milk provided a great amount of magnesium, calcium, and phosphorus with a normal Ca/P ratio in milk as 1.20.

	Goat (per L)	Sheep (per kg)
Calcium (mg)	1260	1950–2000
Phosphorus (mg)	970	1240–1580
Potassium (mg)	1900	1360–1400
Sodium (mg)	380	440–580
Chloride (mg)	1600	1100–1120
Magnesium (mg)	130	180–210
Ca/P (mg)	1.3	1.4
Zinc (μg)	3400	5200–7470
Iron(μg)	550	720–1222
Copper (μg)	300	400–680
Iodine (μg)	80	53–90
Selenium (μg)	20	31

Table 4. Mineral composition of goat and sheep milk (adapted from Balthazar et al. [19]).

As shown in **Table 4**, goat milk is characterized by its lower concentration of iron, zinc, and copper. López-Aliaga et al. [36] reviewed the mineral bioavailability, apparent digestibility coefficients, and the balance of calcium, phosphorus, magnesium, iron, copper, and zinc after the consumption of a goat milk diet compared with bovine milk diet in resected rats. In their work [36], they concluded that based on the particular biological, nutritional, and metabolic characteristics, goat milk can be an excellent natural food in cases of malabsorption syndrome and present a dietary alternative to bovine milk. Although goat milk has a low iron concentration, it has a higher bioavailability than in cow milk due to the presence of higher amounts of nucleotides that in turn increase absorption in the intestine [13].

As far as contaminant metals are concerned, concentrations differ between different studies and sampling (feeding, geographic areas, pollution…), and it is therefore difficult to compare species and breeds. According to Trancoso et al. [35], in goat milk from the main Portuguese indigenous breeds, the values for the potentially toxic elements such as Cd, Pb, Co, and Ni are well below the value stipulated by the Commission of the European Communities Directive EC n° 333 [37] for Pb in milk (0.02 mg/kg). Therefore, consumption of caprine milk does not constitute a risk for human exposure to toxic elements at present in Portugal.

3.5. Vitamins

Goat milk is an adequate source of vitamin A, thiamine, riboflavin, and niacin [11, 36, 38]. However, it presents low levels of folates, as well as vitamin B12, vitamin E, vitamin C, and vitamin D [11, 13] (**Table 5**).

	Goat milk	Cow milk
Fat soluble vitamins		
A		
Retinol (mg)	0.04	0.04
Beta-carotene (mg)	0.00	0.02
D (μg)	0.06	0.08
Tocopherol (mg)	0.04	0.11
Water soluble vitamins		
B1 Thiamin (mg)	0.05	0.04
B2 Riboflavin (mg)	0.14	0.17
B3 Niacin (PP) (mg)	0.20	0.09
B5 Pantothenic acid (mg)	0.31	0.34
B6 Piridoxin (mg)	0.05	0.04
B8 Biotin (μg)	2.00	2.00
B9 Folic acid (μg)	1.00	5.30
B12 Cobalamin (μg)	0.06	0.35
C Ascorbic acid (mg)	1.30	1.00

Table 5. Vitamin content of goat and cow raw whole milk (per 100g) (adapted from Raynal-Ljutovac et al. [13]).

Compared to cow milk, goat milk has lower amounts of vitamin E, folic acid, and vitamin B12, which can result in "goat milk anemia" if additional sources for these vitamins are not present in the diets [13].

In conclusion, nutritionally, goat milk is comparable to cow milk as it contains similar levels of calcium, potassium, phosphorus, and many other nutrients that confer health benefits (see **Tables 1,2,3** and **5**). However, goat milk contains higher levels of 6 out of the 10 essential amino acids than cow milk [39]. It is also richer in monounsaturated and polyunsaturated fatty acids and medium-chain triglycerides [31], while containing less lactose than cow's milk.

The most appealing property of goat milk is its superior digestibility, which can make it particularly helpful in attenuating irritable bowel type symptoms [36].

4. Goat-derived products and nutritional value

The use of goat products was first closely related to a number of medical problems namely food allergies involving cow milk proteins. Cow milk allergy is relatively common during the first 3 years of life. Several studies report that the use of goat milk resolves 30 and 40% of the cases [12].

There are a number of unique physiological and anatomical differences between goats and cows which translate into differences in composition of goat milk and its products [40].

Goat milk products are considered dairy products with greatest marketing potential. Therefore, several characteristics of goat milk are currently the focus of increased research interest [24].

Two glasses (0.5 l) of goat milk or the equivalent amount of cheese or yoghurt can provide up to 94% of the recommended adult daily dietary allowance (RDA) of essential amino acids, 83% of calcium, and 78% of riboflavin needs, while also being a dietary source of other minerals and vitamins, albeit to a lesser extent [40].

4.1. Goat cheese

Literature is exiguous concerning the impact of technology (cheese-making) on every nutrient of cheese, other than fat and proteins. Most studies focus on the gross composition (fat, protein, and lactose contents) of Spanish, Italian, and Greek hard or semi-hard cheeses obtained from small ruminants. Gross composition is mostly dependent on the type of cheese and can be classified according to its dry weight. Although both caprine and ovine milk have been widely used in cheese-making, production of fermented caprine milk using probiotics has not yet been developed, despite the existence of studies showing the requirements for its production. Fermentation increases the nutritional value of caprine milk and improves its flavor, making it more tolerable to the average consumer than raw goat milk.

A review by Haenlein [41] focused on several aspects of yoghurt and cheese goat composition. The benefits for human digestion included proteins with different polymorphisms, forming a softer curd on digestion and cheese-making, and the high content in short chain, medium chain, and mono and polyunsaturated fatty acids.

Regarding cheese produced from goat milk, three categories can be distinguished: traditional cheeses (produced on farms) and prepared mainly for home consumption; cheese produced on farm scale under improved conditions (frequent, for instance, in France, which produces more than 90 varieties of goat cheese) and cheese produced with a mix of sheep and goat milks (produced in all Mediterranean countries, except France) [42].

The production of cheese from goat milk dates back to many centuries. In recent years, the production of cheese from goat milk has acquired commercial advantage in several Western European countries because legislation is not as restrictive for this kind of milk or its products as it is for cow milk products [42, 43]. In France, for example, the production of goat cheese increased approximately 13% (in the same year) while cow cheese increased only 1%. The consumption of goat cheese has been expanding at approximately 20% per year [44].

The composition and characteristics of cheese is highly affected by the characteristics of the milk used in its production. The substitution of sheep milk by goat milk in dairy products is a frequent problem because sheep milk has a higher price. The existence of mixed flocks of goats and sheep might result in accidental or fraudulent mix-ups, affecting profitability as well as the properties and quality of the resultant cheese [45].

Calf rennet was shown to hydrolyze goat casein forming characteristic breakdown of products from individual caseins: β-I to β-V derived from β-casein as well as other primary hydrolyzed products derived αs1-casein; para-K-casein formed from K-casein and other degradation products obtained from αs2-casein. Under the same conditions, both β-casein and αs1-casein present in goat milk seemed to be more sensitive to hydrolysis than their bovine counterparts [46].

Goats milk Gouda cheese is usually made in artisanal units by traditional technology passed on from generation to generation [47] and has a special taste and flavor [42]. However, cheese made under these conditions may not have the minimum hygiene and sanitary standards necessary to obtain a product with minimum quality [48]. In the Eastern Mediterranean, an area favorable to small ruminants, most of the cheese is also produced in small artisanal units, with high temperatures and a lack of refrigeration units. The majority of productions also do not include pasteurization leading to brucellosis, listeriosis, and food poisoning due to enterotoxin production by *Staphylococci*. Therefore, in these areas "white brined cheeses" (WBC), which are ripened and stored under brine until consumption, such as Feta, Domiati, and Beyaz-Peynir, are particularly common and constitute a large share of the cheese market (>50%). Whey cheeses (such as the Myzithra, Manouri, Lor, Anari, Urda, and Skuta) are also traditional products from the Eastern Mediterranean, as the whey obtained from producing sheep's and goat's milk cheese has a very high protein content. The addition of goat or sheep milk or cream to the whey improves the yield of this type of cheese [49].

Karagozlu et al. [50] studied the Cimi Tulum cheeses (made from goats' milk) during 90 days of ripening period. These cheeses contain 57.73% total solids; 30.01% fat; 3.51% salt; 22.27% protein; 2.92% water soluble nitrogen, and 1.75% lactic acid. During the ripening process, the amounts of total solids, fat, salt, protein, water soluble nitrogen, and free fatty acids have all been shown to increase, whereas the salt and fat ratios of the total solid content have

decreased. The percentages of fatty acid composition of these cheeses were 31.73% of oleic acid, 24.19% of palmitic acid, and 9.32% of myristic acid.

El-Sheikh et al. [51] studied the effect of ripening conditions in blue cheese produced from cow's and goat's milk and concluded that blue cheese (Roquefort-style) had similar properties to the ones made from cow's milk.

Queiroga et al. [52] evaluated the nutritional, textural, and sensory characteristics of *coalho* cheese made from goat's (CGM) or cow's milk (CCM) and their mixture (CCGM) during cold storage for 28 days. The choice of milk only seemed to have influenced the moisture, fat, and salt contents of the cheeses. CGM and CCGM showed higher amounts of short and medium-chain fatty acids (such as C6, C8, C10, and C12), and long-chain polyunsaturated fatty acids C18:2n6c. They also showed lower C16 and C16:1 contents. Their properties seem to have been maintained throughout storage time.

Main goat cheese types can be classified as follows [53]:

1. Fresh and soft cheese: Gibna Beida (Sudan), Feta (Greece), and Saint Mareá and Camembert (France). Fresh type is prepared by acid curdling with a small dose of rennet. It is consumed the day after being prepared, and it contains 60–80% moisture. It has a texture similar to Queso-Blanco (Latin America). Soft cheese is produced as fresh cheese, but it is ripened for 10–30 days, and it has 55–60% moisture. For instance, Saint Moreé is surface-ripened cheese prepared by a 24 h coagulation step at 17–20°C after addition of mesophilic lactic culture (20 ml/L) and a rennet solution (6 ml/100L) and has a specific goat cheese aroma and has seven acid compounds present: hexonoic, octanoic, nonenoic, decanoic, 3-methylbutanoic, 4-methyl octanoic, and 4-ethyl octanoic acids; some were already of value in 2 days-old cheese, whereas some others only reached this value after 31.

2. Blue-veined cheese: Savoy (France), Roquefort (France), and Cabrale (Spain). Curd of this cheese is prepared with lactic acid culture and rennet. It has a greenish or bluish marbled appearance after 1–2 hours of curdling and inoculated with penicillium. After salting and piercing, the cheese is ripened for 1–4 months at 9–10°C and 90–95% RH.

3. Semi-hard cheese: Edam (the Netherlands). It contains 40–50% moisture and is prepared by using mesophilic started and rennet. After curdling, cutting the curd, scalting, molding, pressing, and salting, the curd is waxed and ripened for 1–5 months at 8–10°C and 90–95% RH.

4. Hard cheese: Chevrotin (France), Kefalotili (Greece), Ras (Egypt), and Manchego (Spain). It contains 30–40% moisture and is produced in warm countries or in mountainous area. Regarding Chevrotin cheese, it is prepared by culture and rennet curdling. It is preserved for 1–3 months submerged in olive oil.

Hassan et al. [44] fortified goat cheese with caramel, cocoa, and cocoa with walnuts, and these cheeses were prepared in order to be directed to children feeding, given its protein content. The ω-6/ω-3 ratios were at levels 6.0, 7.7, and 4.7, respectively.

The study made during ripening by Niro et al. [54] compared the physicochemical, microbiological, and sensorial characteristics of Caciocavallo cheeses, made from cow milk and a mixture of cow with ewe or goat milk. Different percentages of goat milk added milk to cow milk influenced compositional and nutritional characteristics of these cheeses.

4.2. Goat yoghurt

The preparation of goat yoghurt is made using similar processes to those used in the production of cow milk yoghurt, but it has different organoleptic properties as well as nutritional composition. It presents less viscosity, has softer consistency flavor and higher acidity, during storage [55]. Goat yoghurt has free caproic, caprylic, lauric, and myristic acids. While palmitic and stearic acids were approximately equal, oleic, linolenic, and palmitic acids were lower when compared with cow yoghurt [44]. Regarding amino acid content, goat yoghurt showed about 4 mg/100g of Gly and Pro; 2 mg/100g of Lys, Thr, Ser, Glu, and Ala; and 1–2 mg/100g His, Asp and Leu. Regarding Arg, Val, Meth, and Phe, their concentrations were inferior to 1mg/100g [56].

There are significant changes in gross nutrients between fresh goat milk and yoghurt. Eissa et al. [57] found a decrease in lactose content and pH of the yoghurt after fermentation. Cold storage also resulted in significant changes in gross composition of goat yoghurt. The number of total bacteria and yeast increased significantly within 10 days of storage, decreasing thereafter. Goat milk yoghurt showed in this study lower sensory scores than cow milk yoghurt.

Bano et al. [58] concluded that mixing 75% of goat milk and 25% sheep milk in manufacture of yoghurt improved color, flavor, and texture scores of the resultant yoghurt.

Uysal-Pala et al. [59] showed that drinkable yogurts made from different goat breeds' milk and made with normal and probiotic cultures were evaluated for their sensory characteristics. Yoghurt manufacture with cows and with goats' milk (100, 75, 50 and 25%) substitution blend with cow's milk revealed that goats milk yoghurt (100%) had the highest protein content (4.2%), fat (4.3%) and caproic (c6), caprylic (c8) capric (c10), and total solids (16.2%). Generally, goat's milk yoghurt samples (100, 75, and 50%) were mostly significantly preferred to 25% goat's milk yoghurt sample at ($P > 0.05$).

Al-Abdulkarim et al. [60] studied a sample of dried fermented goat's milk product (Oggtt) obtained from the local market of Riyadh (Saudi Arabia), which was stored for 6 months at 4°C and subjected to chemical composition analysis before and after storage. After storage, total ash decreased nonsignificantly ($P < 0.05$) from 8 to 7.6%, total carbohydrates decreased nonsignificantly ($P < 0.05$) from 35.5 to 33.8%, protein increased nonsignificantly ($P < 0.05$) from 16 to 16.1 g/1, fat content was found to have the same values in all samples before and after storage at 5%, lactose increased nonsignificantly from 28.4 to 29%, acidity decreased ($P < 0.05$) significantly from 0.45 to 0.39%, and pH decreased nonsignificantly from 4.3 to 4%. On the other hand, mineral composition showed ($P < 0.05$) nonsignificant results before and after storage. Ca concentration decreased from 118 to 114 mg/kg and K concentration increased from 185.8 to 188.8 mg/kg. This is a stable product and presents good nutritional value in comparison to daily requirements for healthy human life.

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4.3. Goat butter

The production of butter from goat milk is not very common, and sometimes it is artificially colored in order to look similar to cow butter [61]. There is a difficulty in cream separation, a softer texture, and it presents high tendency to hydrolytic rancidity. Idoui et al. [62] studied a traditional butter from Eastern Algeria. The results showed the presence of lactic acid bacteria (3.51 × 105 + 2.44 cfu/g), psychotrophic bacteria (1.11 × 105 + 1.31 cfu/g), moulds and yeasts (39.08 × 102 cfu/g), lipolytic bacteria (4.41 × 103+5.91 cfu/g) and the absence of total coliforms except in one sample. An analysis of fatty acids was made by GC-MS that showed a prevalence of saturated fatty acids, namely palmitic acid with a low rate of unsaturated fatty (oleic acid).

4.4. Goat meat

Goats are animals with fairly low-fat content. Several authors have indicated that the fat content of goats is 47–54% lower than that of cattle and sheep. The introduction of goat meat in diet may become an important measure for the prevention of cardiovascular diseases

Banskalieva et al. [63] also pointed out that further experimentation is needed to characterize interactions between factors such as race, age, and nutritional status in the lipid profile of goat for a better understanding of their meat quality. Little is known about the lipid profile of goat meat, but some studies indicate that oleic, palmitic, stearic, and linoleic are the predominant acids in muscles. Rhee [64] reported that goats have higher concentrations of desirable fatty acids than cattle and sheep, but at levels similar to lean meat of pigs.

Several factors such as race, gender, stress, environment, management, diet, weight, and health condition affect the chemical composition of goat meat. Studies have shown that the average composition of meat obtained from Serbian white goats to be estimated around 75.42% water, 3.55% fat, 19.95% protein, and 1.06% minerals, whereas meat obtained from Balkan goats had a similar composition, with a water content of 74.51%, 3.92% fat, 20.55% protein, and 1.04% minerals. The energetic value was similar in both breeds and is around 580 kJ per 100 g of meat [65].

The quality of goat meat is influenced by its water content. The muscles contain approximately 75% water, which is distributed within the myofibrils, between themselves, between the cell membrane (sarcolemma), and between the muscle bundles. The cooling or freezing mode after slaughter, especially during the rigor, is of great importance for the percentage of water that will remain in the meat.

Adipose tissue of slaughtered animals contains 50–95% fat, 3–35% water, 2–15% protein, and 0.1–0.6% mineral matter. The composition of adipose tissue is highly variable and depends of nutritional status, breeding, age, and type of animal. In each cell, there are 40–50 types of fats, which represent about 5% of the organic matter present in cells. The amount of fat per cell varies from tissue to tissue, for instance, cells of the nervous system are extremely rich in fats. Diet influences the deposition of fat in the muscular tissue, as well as the saturated (SFA) and polyunsaturated fatty acid (PUFA) concentrations in cattle.

In ruminants, lipids from meals suffer hydrolyzation and biohydrogenation processes in the rumen, resulting in absorption of saturated fatty acids in the digestive tract. This fact could help explain the higher percentage of saturated fats in meat products obtained from ruminants. Several studies have been conducted in an attempt to determine the fatty acid profile of goat meat [66]. However, the role of certain processes such as biohydrogenation, transition of unsaturated into saturated fatty acids, elongation of fatty acid chains, metabolism, and deposition rate are yet to be fully understood.

The composition of the fat in goat meat and other ruminants differs from that of monogastric animals, having larger amounts of SFA and lower quantities of PUFA, with C18:1 and C18:2 *trans* and *cis* isomers of FA are also present in goat meat. In animals, the main PUFA (C18:2n-6 and C18:3n-3) are obtained from the diet. However, in ruminants, these products suffer biohydrogenation processes in the digestive tract, originating saturated fats as well as other intermediate products, which include *cis* and *trans* C18:1 isomers and C18:2 *trans* isomers, conjugated or unconjugated [67]. While grain feeds are a food source of C18:2n-6, green grass on pastures are richer in C18:3n-3 [68], which is more desirable as it could lead to higher contents of omega-3 fatty acids in meat products.

Meat products derived from ruminants are a dietary source of CLA. C18:2 *cis*-9, *trans*-11 is the most frequent isomer of CLA, and is also present in higher amounts in the meat of ruminants fed on pasture than in the meat of ruminants fed with grain. Despite the fact that a fraction of this fatty acid occurs in the rumen, about 70–80% of the acid present in the tissues results from endogenous transformation C18:1 *trans*-11 by the enzyme Δ9 desaturase [69]. Therefore, the difference in CLA concentration in the tissues results mainly from the amount of C18:1 *trans*-11 absorbed in the rumen.

5. Nutrient functionality

Nowadays, nutrients have emerged as an important research topic in food and nutrition sciences as they appear to be able to modulate the inflammatory status of humans [70]. Dairy products represent a particularly interesting food type to study in the context of inflammation, mostly because of milk's ability to support the development of the immune system of the newborn, to inhibit bacterial growth, and to provide anti-oxidative and anti-inflammatory protection [71]. Some of these properties might still be maintained in the context of the consumption of dairy products by human adults. Additionally, the ability of milk and milk-products to deliver supplements to the human organism able to modulate the gut microbiota, a key regulator of immunity, is another factor which might help influence immune and inflammatory processes [72, 73].

Recently, reviews by Hsieh et al. [74, 75] have described the effects of different bioactive components of milk and dairy products in preventing low-grade systemic inflammation and in acting as coadjutants in conventional therapies.

In the following sections, after a brief overview structure and function of the immune system, we will focus on the effects of several bioactive compounds found mainly in goat milk and their effects in low-grade systemic inflammatory diseases and immunity.

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5.1. Overview of immune system: low-grade systemic inflammation and gut-systemic inflammatory associations

Inflammation is one of the main biological processes involved in response to potentially detrimental stimuli to the body and can be classified as acute or chronic with different processes involved in each of these types. Acute inflammation is an immediate and short-lasting response to irritation, injury, or infection which leads to the activation of mechanisms such as increased blood flow, greater blood vessel permeability, and movement of white blood cells to the affected site. These mechanisms are responsible for the classic signs of inflammation: redness, edema, heat, pain, and decreased function [76]. Chronic inflammation is a long-lasting response to factors such as poor nutrition, stress, environmental toxins, and processes related to aging [76]. These prolong the inflammatory response, leading to destructive reactions which, coupled with inappropriate repair processes, eventually lead to the clinical symptoms of disease [77].

The human immune system possesses innate or nonspecific and adaptive mechanisms that work synergistically to protect the body against injury and infection. Innate immunity constitutes the first line of defence, providing immediate response albeit unspecific response to localized injury or invasion by an infectious agent. Innate immunity is triggered when the inflammasome (a large sensor protein produced by bone marrow) detects a toxic substance and stimulates the production of macrophages to destroy harmful stimuli. Macrophages are able to recognize different types of pathogens and are able to react either by producing several mediators that activate other elements further downstream inflammatory cascade (these include, for instance, toll-like receptors, cytokines, or transcription factors); or by eliminating them directly through a process known as phagocytosis. Innate immunity, however, has a limited duration and is not able to stop all pathogenic stimuli. When overtaxed, the body's adaptive immunity mechanisms are activated [78].

Adaptive immunity or acquired immunity is based in highly specialized responses directed at specific antigens [77]. It can be divided into two types: humoral immunity, in which the B lymphocytes, produced in the bone marrow, generate antibodies targeting specific antigens present in the pathogen in question; and cell-mediated immunity, in which T lymphocytes, matured in the spleen and lymph nodes, recognize antigens present on infected cells and lead to their destruction. Memory cells are also a part of the adaptive immunity response and recognize and react to repeated exposures to specific antigens [77].

When, in the human body, the mechanisms of innate and adaptive immunity are ineffective in eliminating a harmful stimulus, illness occurs. Normal function of the cells is disrupted by processes that include leukocyte proliferation, oxidative reactions, and fibrosis caused by repeated or uncontrolled inflammatory responses. A chronic low-grade inflammatory state as a pathological feature of a wide range of chronic conditions, such as metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM), atherosclerosis, cardiovascular diseases (CVD), cancer, neurological diseases, among others, has been recognized [79–81]. The numbers of illnesses, which are related to molecular mediators of inflammation, are large and expanding.

Inflammation constitutes one of the basic mechanisms of the innate immune response. In general, inflammation is a local response to cellular injury that aims not only to eliminate the toxic agents but also to promote repair of damaged tissue [78].

The microbiota present in our bodies also plays an important yet often under looked part in maintaining systemic metabolism and cardiometabolic health [82, 83]. Increasing evidence indicates that a relationship between microbiota, the immune system, and inflammatory processes exists. When chronic disease (such as obesity or processes related to aging) occurs, microbiota loses "richness" altering gene expression diversity and increasing low-grade chronic inflammation [83]. According to Kurashima et al. [84], gastrointestinal tract-microbiota interactions influence immune function by maintaining the function of the mucosal immune system, protecting against invasion by pathogens, and maintaining the integrity of the barriers present in gastrointestinal tract. The permeability of the walls of the gastrointestinal tract to the lipopolysaccharides (LPS), found on the outer membrane of Gram-negative bacteria, can also induce low-grade systemic inflammation, as LPS is a powerful proinflammatory. In the elderly, a higher count of bacteria that produce LPS in the colon, coupled with a lower amounts of bifidobacteria [85], increases gut permeability, leading to higher amounts of LPS entering the bloodstream, which in turn aggravates inflammation [86]. One of the mechanisms through which LPS may be an important trigger in the development of inflammation and metabolic diseases is an interaction with the Toll-like receptor 4 present on the surface of mononuclear cells [87]. Moreover, in addition to its role in low-grade systemic inflammation, emerging evidence suggests that the gut microbiota can also have an influence in the risk of high-grade autoimmune inflammatory conditions such as type 1 diabetes mellitus, celiac disease, inflammatory bowel disease, and rheumatoid arthritis [88–90].

5.2. Food allergy

Specific immune response also plays a lead role in food allergy. The term "allergy" can be used to define an abnormal adaptive immune response directed against noninfectious environmental substances (allergens), including noninfectious components of certain infectious organisms. After antigen contact, the body responds with an excessive reaction (IgE antibodies, histamine release). The symptoms are dramatic and acute. In allergic disorders, such as some food allergies, these responses are characterized by the involvement of allergen-specific IgE and T helper 2 (T_H2) cells that recognize allergen-derived antigens [91].

Recent studies suggest that the consumption of dairy products is inversely associated with low-grade systemic inflammation [92]. The cross-sectional nature of these studies precludes definite conclusions on the cause-and-effect relation between dairy food consumption and inflammatory outcomes. Considering distinctive proprieties of goat milk, we will summarize some of the bioactive compounds present in goat's milk and dairy products and their effects on health.

6. Anti-inflammatory effects of goat milk and its derivatives

6.1. Bioactive peptides

Bioactive peptides (BP) have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health [93]. According to Atanasova and Ivanova [94], goat milk is as "close to perfect food as possible in nature."

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As we have previously described, the protein present in goat milk is comprised of about 80% caseins and 20% whey proteins. Some of the peptides and proteins in milk present direct biological activity, while other proteins have a latent biological activity, which is activated only upon proteolytic action. For example, the active forms of caprine calmodulin (calcium-binding protein) are the soluble C-terminals obtained as a by-product from the action of chimosin on k-casein during the milk clotting process of cheese-making. These peptides are also important sources of bioactive ACE-inhibitory and anti-hypertensive peptides [95, 96]. Moreover, goat milk possesses other minor proteins including immunoglobulins, lactoferrin, transferring, ferritin, protease peptone, prolactin, and folate-binding protein with biological activity.

Furthermore, a variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt and cheese. The main bioactive peptides of goat milk and its derivatives will be described below.

6.1.1. Biopeptides that lower blood pressure

Several epidemiological studies have linked dietary intake of milk and dairy foods with a decreased risk of hypertension [97]. Their high mineral content (in calcium, potassium, and magnesium) and certain proteins present in these products (as well as their hydrolysates) have been thought to be involved in the anti-hypertensive effect of these products [98]. Angiotensin-converting enzyme (ACE) is a multifunctional enzyme that acts as one of the main regulators of blood pressure. ACE inhibition, restrain the formation of angiotensin II or block its receptors leading to arteriolar vasodilation and a reduction of total peripheral resistance. Therefore, ACE inhibition is currently considered as one of the best strategies for hypertension treatment [99].

Inflammation and oxidative stress play an important role in the pathophysiology of hypertension; however, the description of the mechanisms involving all interplays between them is behind the scope of this chapter, nevertheless, we might consider the link between angiotensin II and immune system. Nosalski et al. [100], in a recent revision, state that the bulk of mechanistic model studies clearly indicate that a complex network of interactions between T cells, dendritic cells, monocytes, and B cells may be involved in hypertension. The lack of this immune response has a blunt response to angiotensin II-simulated hypertension and may promote hypertension by potentiating vascular dysfunction. Consequently, hypertension is associated with significant activation of immune and inflammatory systems and shares several functional differences with other immune-mediated diseases [101, 102].

Several studies have been done to investigate the bioactivity of goat milk protein hydrolysates and the release of ACE-inhibitory and anti-oxidant peptides with individual proteases such as thermolysin, trypsin, subtilisin, papain, and pepsin or their combinations [95, 103]. Espejo-Carpio et al. [95] and De Gobba et al. [104] identified many casein-derived peptides from hydrolyzed proteins of goats' milk, which were enzymatically liberated by a combination of subtilisin and trypsin. Among them, many peptides contained tyrosine in their sequence and had anti-oxidant and ACE-inhibitory activities. More recently, Ibrahim et al. [105] have shown that ACE-inhibitory peptides can be released from goat milk caseins and whey proteins after gastric pepsin digestion. In their study, they found one peptide from whey

β-lactoglobulin, PEQSLACQCL and two peptides from caseins, ARHPHPHLSFM (fragment 96–106 κ-casein), and QSLVYPFTGPI (fragment 56–66 β-casein). These peptides displayed ACE-inhibitory activity that compares favorably with the activity of anti-hypertensive drugs with ACE-inhibitory action. These peptides as well as other hydrophobic peptides additionally exert anti-oxidant and anti-inflammatory activities. Therefore, goat milk and goat whey bioactive peptides present anti-hypertensive activity through inhibition of ACE, but considering all the mechanisms involved in the pathophysiology of hypertension, the existence of multifunctional peptides must be considered [106].

6.1.2. Antimicrobial peptides

The antimicrobial activity of milk is mostly attributed to the presence of immunoglobulins and other proteins, such as lactoferrin, lactoperoxidase, and lysozyme. It is generally accepted that the total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin defensive proteins. This might be because naturally occurring proteins and peptides act synergistically with peptides that result from metabolization of inactive protein precursors [107]. The proteins present in milk proteins have been proven to act as antimicrobial peptide precursors, thus improving the ability of natural defences to eliminate invading pathogens. Consequently, food proteins can be considered components of nutritional immunity [94]. A paper by Budiarti et al. [108] demonstrates the presence of Alpha-S2 casein in Ethawah goat milk and yoghurt. This protein contains eight bioactive peptides, with different effects, such as anti-osteoporotic or anti-inflammatory effects, and it was not found in cow fresh milk [109, 110]. More recently, Triprisila et al. [111] reported the effect of antimicrobial activity from CSN1S2 protein as a member of casein protein from Ethawah breed goat milk and yoghurt against pathogen Gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus,* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi,* and *Shigella flexneri*). The results of their work showed a greater inhibitory effect on Gram-positive bacteria than on Gram-negative bacteria. They also demonstrated that milk has a higher antimicrobial activity than yoghurt [111].

Goat lactoferrin has been studied after the beneficial effects demonstrated by both human and bovine lactoferrin. The levels of this protein in sheep and goat milk are slightly higher than in cow milk with values of approximately 0.107 ± 19 mg/mL [112]. The concentrations of lactoferrin in goat milk during various stages of lactation have been shown to vary in direct proportion with the number of somatic cells present in the milk samples. These parameters are influenced by a number of physiological processes [112]. Another study, which compared the glycosylation of goat milk lactoferrin with that of other glycoproteins present in human and bovine milk, demonstrated similarities in glycans present in both human and goat milk samples. However, some novel glycans were also identified goat milk, which did not exist in the human milk samples [113]. Considering its high digestibility, immunological properties, and high mineral concentration as well as the similarities between human and goat lactoferrin, goat milk can be considered an attractive candidate for use in infant formula supplementation. Lactoferrin plays an important role in both innate and adaptive immunity responses. Not only pathogens have high affinity toward the iron present in lactoferrin, it also induces changes in leukocytes, involved in innate immune system, by increased activity of NK cells and increasing the phagocytic activity of neutrophils and macrophages [114].

In the same way, caseins seem to be good antimicrobial peptides against Gram-negative bacteria [115]. Esmaeilpour et al. [116] investigated the antimicrobial action of goat milk casein hydrolysates produced by the proteolytic enzymes trypsin and ficin and by combination of both enzymes. The authors obtained fractions with significant antimicrobial activities against Gram-negative and Gram-positive bacteria.

6.1.3. Cytomodulatory and anticancer peptides

Goat milk lactoferrin demonstrates not only to possess antimicrobial action but also to induce apoptosis in a human cervical cancer cell line [117]. Considering the resemblance with other lactoferrins, goat lactoferrin might be able to present protective activity against other cancers. However, the research in this area is only in its infancy. The mechanisms by which this molecule might exert its activity were recently reviewed by Zhang et al. [118]. These properties of lactoferrin constitute an interesting alternative to chemoprevention, and the currently used anticancer drugs. In addition, its stability through the gastrointestinal tract is beneficial if oral administration is envisaged.

It seems that intact and hydrolyzed goat whey protein concentrates, blood serum albumin, and skim milk inhibited lymphocyte proliferation in dose-dependent reactions [119]. Su et al. [120, 121] reported that an anticancer bioactive peptide (ACBP) extracted from goat spleens significantly inhibited the growth of human gastric cancer line BGC-823 in vitro in a dose-dependent manner. In vivo, ACBP-inhibited human gastric tumor growth in a *xenograft* model with no apparent cytotoxicity to host. The study suggested that ACBP could be a powerful anticancer biological product through induction of cell apoptosis and cell cycle arrest. Furthermore, Yu et al. [122] found using in vitro and in vivo samples that the bioactive peptide-3 (ACBP-3), a peptide isolated from goat liver, presented antitumor properties on gastric cancer stem cells (GCSCs). ACBP-3 was also found to decrease CD44 (+) cells and suppress proliferation of SC (spheroid colonies) cells and their clone-forming capacity alone or in combination with cisplatin, in a dose-dependent manner [122].

6.1.4. Antioxidant peptides

Antioxidant peptides are particularly interesting because they can potentially prevent or delay oxidative stress as well as low-grade systemic inflammation associated chronic diseases [74, 94]. In this sense, milk proteins as well as milk-derived proteins have been considered as potential carriers for the delivery of antioxidant peptides in the gastrointestinal tract, where they may exert direct protective effects by scavenging reactive oxygen species and reducing the oxidative stress [123]. Only a few studies on the antioxidant properties of goat milk protein-derived peptides have been performed. Silva et al. [124] identified three antioxidant peptides in a water extract from a goat cheese-like system made using an extract from *Cynara cardunculus*. Nandhini et al. [125] found that goat milk fermented with *Lactobacillus plantarum* had potent radical scavenging and lipid peroxidation inhibition activity, although they did not identify the active peptides. Li et al. [126] identified antioxidant peptides in a goat's milk casein hydrolysate made using two enzymes (alcalase and pronase), although some of which did not match with the goat casein sequences available in protein databases. De Gobba et al. [104] isolated and identified antioxidant peptides formed from goat's milk protein fractions

by enzymatic hydrolysis using trypsin and subtilisin, both individually and in combination. They found hydrolysates with high radical scavenging activity attributed to the presence of short peptides. Furthermore, an abundance of tyrosine in novel casein-derived peptides seems to play an important role in the radical scavenging capacity of the peptides. Also, peptide fractions with a high abundance of phenylalanine showed the ability to prevent the formation of secondary lipid oxidation products.

6.1.5. Immunomodulatory/anti-inflammatory peptides

As stated above, some peptides can present more than one function. Antimicrobial action of goat lactoferrin and some goat caseins is not only antimicrobial but also acts in the immune system.

A proline-rich polypeptide complex, named colostrinin (CLN), was isolated from ovine colostrum [127]. Cell culture and in vivo studies have shown colostrinin to have anti-inflammatory and antioxidant properties. It also was found to play a regulatory role in growth and differentiation of lymphocytes. Colostrinin also inhibits pathological conditions associated with β-amyloid aggregation in neurons [128, 129]. The CLN complex was also found to present neuroprotective activity, inhibiting nerve cell apoptosis induced by the deposition of toxic amyloid [130]. Colostrinin improves learning and memory in rats, delays the progression of dementia, and loss of long-term memory in aging animals [131, 132]. In humans, positive results of preliminary clinical trials have been found [133, 134].

Zhang et al. [135] also found DPP-IV-inhibitory peptides in goat milk casein-derived hydrolyzed with a combination of trypsin and chymotrypsin thus conferring moderate antidiabetic properties to goat's peptides.

The works of Jirillo et al. [136] recently investigated the effects of goat milk on human blood cells in terms of cytokine release and nitric oxide (NO). The results of their work demonstrated that goat milk was able to trigger cytokine production (IL-10, TNF-α, and IL-6) as well as activate NO release from blood cells. It is well known that NO release can be useful in the prevention of cardiovascular disease being a strong vasodilator and an effective antimicrobial agent. Furthermore, NO possesses other antiatherogenic activities, such as (i) inhibition of influx of atherogenic monocytes and LDL into the wall of arteries; (ii) inhibition of adhesion to the vascular wall of proliferating smooth muscle cells; (iii) inhibition of platelet aggregation; (iv) inhibition of the expression of genes involved in atherogenesis [137]. Goat milk can be helpful in maintaining inflammatory homeostasis by stimulating production of multiple cytokines with a variety of effects, such as TNF-α (a proinflammatory cytokine), IL-6 (an acute phase reactant and growth factor for B cells), and IL-10 (an anti-inflammatory cytokine) [138].

Nowadays, the modulator effect of the diet on the gastrointestinal tract functions has been accepted as essential for maintaining and improving the general health of the host [139]. Dairy proteins, hydrolysates, and peptides have been demonstrated to transform the dynamics of mucus mainly via influencing the mucin secretion and expression and the number of goblet cells. The β-casein-derived peptide β-casomorphin 7 produced the same effects that have

been suggested to be mediated by interaction with opioid receptors [140]. Also, this peptide, another β-casein fragment (identified in commercial yoghurt), whey proteins hydrolysates and β-lactorphin have been reported to stimulate the expression of mucin Muc2 and Muc3 genes [141, 142]. Additional gut-protective effects, exerted by a cheese whey protein diet and a diet supplemented with Thr, Ser, Cys, and Pro residues, have been demonstrated by Sprong et al. in colitis and chemical-induced ulcerative gastric lesions [143, 144]. The enhancement of the mucosal immune response is also a dietary modulating strategy of the defense systems protecting the gastrointestinal tract. Kitamura and Otani showed that ingestion of cakes enriched with casein phosphopeptides increased fecal IgA content in healthy individuals, suggesting a positive effect on mucosal immunity [145].

Imbalances in both oxidative and inflammatory status are involved in the etiology of several human chronic diseases that affect the digestive tract, such as ulcerative colitis and Crohn's disease. This has encouraged the search of natural preventive treatments against these imbalances and, consequently, against disease [146].

The minor constituents of goat milk, namely lysozyme and transforming growth factor-β (TGF-β), seem to offer additional protection against intestinal cell damage/inflammation [147]. Several authors have shown that oral administration of TGF-β has anti-inflammatory effects at the intestinal level in animal models of colitis [148]. Schiffrin et al. showed that TGF-β administration lowered leucocytes in the blood stream, as well as the levels of the acute phase reactants fibrinogen and orosomucoid. Colonic weight and thickness and mRNA synthesis for IFN-γ production were also reduced. Finally, TGF-β supplementation also increased mucin-2 production in the caecum and normalized muscle proteolytic activity [149]. Goat milk has a much higher level of growth factor activity than that of cow milk [140], thus making goat milk a possible nutraceutical for gastrointestinal disorders [150].

6.1.6. Prevention of milk allergy

Cow's milk allergy is a common disease of infancy and childhood, and its prevalence is about 2.5% during the first 3 years of life [151]. It is an IgE-mediated allergy, meaning that body produces IgE antibodies against certain protein (allergens) in cow milk. Repeated ingestions of milk lead to identification of antigens present in milk by these IgE antibodies, triggering an immune response that causes symptoms such as eczema, respiratory symptoms (wheezing or asthma), gastrointestinal symptoms, or anaphylaxis. Some proteins, namely αS1-casein and β-lactoglobulin (the structures and composition of which vary between animal species) are known to be important allergens in cow's milk allergy. The allergy-causing properties of β-lactoglobulin can be partially eliminated by heat denaturation [11]. However, caseins maintain the capability of binding to lgEs even after a strong denaturing process [39]. Because the content of αS1-casein is low in goat milk, it is plausible that for children with high sensitivity to αs1-casein of cow milk, goat's milk could be considered an alternative source of milk. More recently, several studies report extensive cross-reactivity between cow and goat milk, caused by cow milk-specific IgE antibodies [152]. Because of the cross reactivity, all scientific reports dissuade persons with cow milk allergy to ingest goat milk. Interestingly, allergy to goat's and sheep's milk without allergy to cow's milk has been reported [153]. However,

this food allergy develops later in childhood and not in early childhood as cow's milk proteins allergy, and all the children tolerated cow's milk. Cow milk proteins have been shown to have higher binding capacity to lgE and lgG than proteins present in goat milk. In animal models, cow milk has induced higher lymphocyte proliferation, IL-4 production, histamine secretion, and IgG production [154], which indicates a more exacerbated allergic response.

As described above, peptides present in goat products have been claimed to be active on a wide spectrum of biological functions or diseases, including blood pressure and metabolic risk factors (coagulation, obesity, lipoprotein metabolism, peroxidation, gut and neurological functions, immunity, and cancer). **Figure 1** presents a schematic representation of these different active biological functions.

6.2. Bioactive lipid components of goat milk

Milk lipids have a complex composition, consisting of a variety of bioactive substances with various health effects. Lipids in milk are organized in unique structures: milk fat globules. These structures are important in delivering essential nutrients to the neonate, as they increase bioavailability of lipids. Fat globules have also recently an object of interest in food science, namely their use as a method of delivering other beneficial bioactive nutrients. As previously

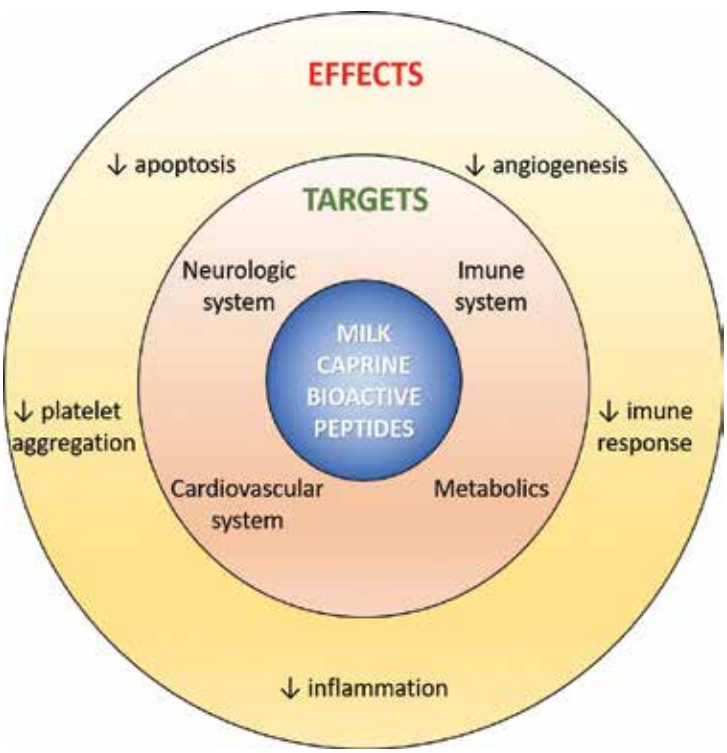

Figure 1. Schematic representation of the wide spectrum of biological functions of caprine biopeptides.

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described, caprine milk has more fat globules than cow milk, with a smaller size. These characteristics are important, as they increase the surface area of the globules in contact with pancreatic lipase in the intestinal tract. This allows for better digestibility and a more efficient lipid metabolism, when compared with cow milk fat [155]. Goat milk is therefore recommended for infants, elderly, and convalescing people.

There are several lipid components that have bioactive functions, such as short- and medium-chain fatty acids (MCT), phospholipids, cholesterol, gangliosides, and glycolipids, etc. Goat milk has a relatively high amount of saturated fatty acids (SFAs; 53–72%) and a relatively low content of polyunsaturated fatty acids (PUFAs; 2–6% of fatty acid composition); the remaining fat is constituted by monounsaturated fatty acids (MUFAs) [156].

Table 3 demonstrates that the fatty acid content of goat milk differs from that of cow milk, the former having much higher amounts of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2) acids, but lower stearic (C18:0) and oleic acids (C18:1) contents. Three of the medium-chain fatty acids (caproic, caprylic, and capric) represent 15% of the total fatty acid content, compared to only 5% in cow milk [12].

According to Ceballos et al. [26], goat milk also has higher proportions of n-3 and n-6 polyunsaturated fatty acids (PUFA) as well as conjugated linoleic acid (CLA). The work of De La Fuente et al. [157] demonstrates that flock, day of testing within each flock, lactation stage, age of ewe, and season had a significant effect on fatty acid content in dairy sheep milk. They also show that the three quantitatively most important sources of variation in fatty acid content were flock, season, and circumstances of testing within each flock. The most important variations related to testing within each flock and season effects occurred in rumenic (CLA) and linolenic acids. These two fatty acids were higher in spring-summer than in winter. Moreover, lactation stage and age of ewe had a significant effect on some FA. As the age of ewe increased, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decreased and sum of short-chain saturated fatty acids (C4 to C10, SCFA), sum of medium-chain saturated fatty acids (C12–C15, MCFA) increased. Despite the fact that the work of De La Fuente et al. [157] focused on ovine milk, the same factors can also influence goat milk.

Conjugated linoleic acids (CLA) are naturally occurring isomers of linoleic acid. They are a result of biohydrogenation in the rumen of ruminants and influence the concentration of fatty acids in milk [29]. The conjugated linoleic acid found in goat milk fat comes from two sources: ruminal biohydrogenation of linoleic acid (C18:2 n-6) that leads first to vaccenic (trans-11 C18:1) and finally, to stearic acid (C18:0); and synthesis from trans-11 C18:1, an intermediate product of biohydrogenation of unsaturated FA, in animal tissues. Therefore, the conjugated linoleic acid contents in food products derived from ruminants are derived from incomplete biohydrogenation of unsaturated fatty acids in the rumen. Ruminal biohydrogenation, combined with the mammary lipogenic and Δ-9 desaturation pathways, modifies the profile of dietary fatty acids and consequently milk composition [29]. Food products obtained from ruminants, such as milk, cheese, and meat, contain more CLAs than foods of nonruminant origin [158].

Many researchers have investigated the effect of goat milk fatty acids in human research. The short- and medium-chain fatty acids in goat milk exhibit several bioactive effects in digestion, lipid metabolism, and treatment of lipid malabsorption syndromes. The important bioactive lipid components in goat milk have been explored in a recent review by Park [140].

Short- and medium-chain fatty acids are transported directly from the intestine to portal circulation after hydrolysis of the parent triglyceride, without undergoing resynthesis of triacylglycerols. Therefore, they can be used for instant energy production for muscles, heart, liver, kidneys, blood platelets, and the nervous system and are not available for adipose formation. The consumption of a wide range of fermentable carbohydrates can lead to the synthesis of butyric acid, a well-known modulator of genetic regulation, by endogenous microbiota in the lower intestine. This fatty acid is also present in milk, and its properties have prompted various investigators to target this mechanism in an attempt to reduce cancer risk [159]. Butyric acid, by binding short-chain fatty acid receptors, also manages to reduce the inflammatory response in the intestine [160].

Capric and caprylic acids have similar antimicrobial effects. Caprylic acid lowers salmonella infection in chickens [161] and both caprylic and capric acid have antiviral activity. Monocaprin, their monoglyceride form, has been shown *in vivo* to possess antiviral activity against infection by retrovirus [162]. The release of lauric acid in the stomach may have direct antimicrobial activities toward *Helicobacter pylori* [163]. The above fatty acids do not increase blood lipid levels, and they do not contribute to the risk of obesity. Palmitic acid has shown to improve intestinal absorption not just of the palmitic acid but calcium as well as stimulated the expression and activities of the transcription coactivator PGC-1b and by so doing promoted the transcriptional regulation of biosynthesis of lipoproteins from the liver [164, 165].

Milk fat contains essential fatty acids (EFAs) that are not synthesized by the body and therefore have to be supplied with food. Polyunsaturated fatty acids have beneficial health effects by increasing the synthesis of eicosanoids, molecules that regulate cardiovascular function, blood pressure, coagulation, plasma triacylglycerol concentrations, immune response, inflammation, neoplastic proliferation, hormone, and neurotransmitter activity, gene expression, renal function, and pain. Eicosanoids boost immunity, lower cholesterol levels in peripheral blood by stimulating lipid transport, thus reducing the risk of ischemic heart disease [166]. Fatty acids from the n-3 family, commonly known as omega-3 fatty acids, can be useful in the management of inflammatory diseases, such as rheumatoid arthritis, as well as in decreasing symptoms of mental disorders and dementia. DHA was found to be effective in late-stage Alzheimer's disease [167].

Conjugated linoleic acid was recognized as having anti-oxidative and anti-carcinogenic properties in animal model studies [158]. CLA has important functional properties, such as inhibiting the growth of skin, gastric, breast, and colorectal cancer cells [168]. The *cis*-9, *trans*-11 isomer shows the biological activities, but other isomers seem to have beneficial effects as well, such as *trans*-10, *cis*-12, which is believed to prevent the development of obesity [169]. CLA helps prevent osteoporosis, reduces blood sugar levels, boosts immune system function, lowers total cholesterol and LDL cholesterol levels, and improves the LDL/HDL ratio in the blood plasma, thus contributing to the prevention of ischemic heart disease and atherosclerosis [170].

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Furthermore, sphingolipids and their digestion products, ceramides and sphingosines, have been associated with a variety of health benefits, namely anti-carcinogenic effects, immune regulation, prevention of food-borne infections, and reduction of serum LDL cholesterol [170].

As it was described above, milk is not only the main source of energy for the neonate of each species, but its components also have the potential to influence many aspects of physiology from the central nervous system to the immune system, while also exerting both antibacterial and antiviral effects.

6.3. Biological activity of oligosaccharides

Lactose is the major carbohydrate in milk with an average content of 4.1 g/100 mL in goat milk [11]. This disaccharide is a valuable nutrient because it favors the intestinal absorption of calcium, magnesium, and human milk oligosaccharides phosphorus, and the utilization of vitamin C. On the other hand, milk oligosaccharides possess prebiotic and anti-infective properties. The amount of oligosaccharides in caprine milk ranges from 250 to 300 mg/ml. Sialic acid, a general name for N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid 355 (Neu5Gc), exists in many of these oligosaccharides and is important in promoting the development of the brain during infancy, among other beneficial effects [140]. Although goat milk is the richest source of oligosaccharides among the different types of milk from farm animals, its content is significantly lower compared to human milk (21–24 g/l for human colostrum; 12–13 g/l for mature human milk) [11]. However, from a structural point of view, among the oligosaccharides found in different types of milk, goat milk oligosaccharides are the most similar to human milk oligosaccharides [171].

While a wide range of biological functions has been attributed to human milk oligosaccharides, less information is available regarding the biological activities of ruminant's milk oligosaccharides and complex oligosaccharides. In short, human milk oligosaccharides are considered to have mainly prebiotic and anti-infectious properties, thus being beneficial for humans, especially for the human-milk-fed neonate [172]. Goat milk seems to be a very appealing candidate for a natural source of human-like oligosaccharides due to its concentration and structure. However, research data on the bioactive oligosaccharides of goat milk is still scarce.

Based on the above, goat oligosaccharides have gained lately much attention as potential nutritional supplements or therapeutic agents. Therefore, they have been used in a number of studies in order to evaluate their health-promoting effects.

6.3.1. Prebiotic and antipathogenic activity

A healthy gut microbiota, composed primarily of bifidobacteria and lactobacilli, which are bacteria presenting saccharolytic activity, has been linked with multiple health benefits. This can be explained by the interactions of intestinal bacteria with metabolic processes and the endocrine and immune systems of infants and adults. Intestinal colonization with a balanced microbiota is of major importance for the appropriate development of the immune system, and there is an enormous scientific and commercial interest in modifying the microbiota for health promotion [173]. As the gut is sterile at birth, it is an organ sensitive to environmental

influences. Furthermore, there is an intensive crosstalk between gut microbes and the intestinal epithelium throughout life [174, 175]. The species present in intestinal microbiota, once established, appear to be difficult to modify. Two strategies have been developed to promote a healthy microbiota: the administration of live bacteria (probiotics); or substances (prebiotics) which pass through the gastrointestinal tract undigested, but constitute substrate for desirable bacteria in the gut. The ability of saccharolytic bacteria to break the bonds present in carbohydrates make them able to use these substances to proliferate in detriment of other bacteria which do not possess enzymes to degrade these products. These bacteria provide additional benefits by producing important nutrients, such as vitamins, amino acids, or short-chain fatty acids, which can then be absorbed by the host. The mechanisms by which the intestinal mucosa perceives and responds to microbes, both pathogenic and commensal, are not completely known yet. Based on the literature and considering the analogy between human and goat milk oligosaccharides we might hypothesise a goat oligosaccharides metabolism and potential functions (**Figure 2**).

One of the main features of oligosaccharides is that they can only be consumed by very specific bacteria strains that possess the appropriate set of enzymes to cleave their complex structure. This prebiotic effect is associated with improved health outcomes because it allows specific changes, both in the composition and/or in the activity in the gastrointestinal microflora [176]. Considering that oligosaccharides are only partially digested in the small intestine, they can reach the colon intact where they selectively stimulate the development of lactobacilli and bifidobacteria. Oliveira et al. [177] evaluated the prebiotic activities of the natural oligosaccharides recovered from caprine milk whey and reported that the natural oligosaccharides from caprine milk whey favored the development of *Bifidobacterium* spp. and produced

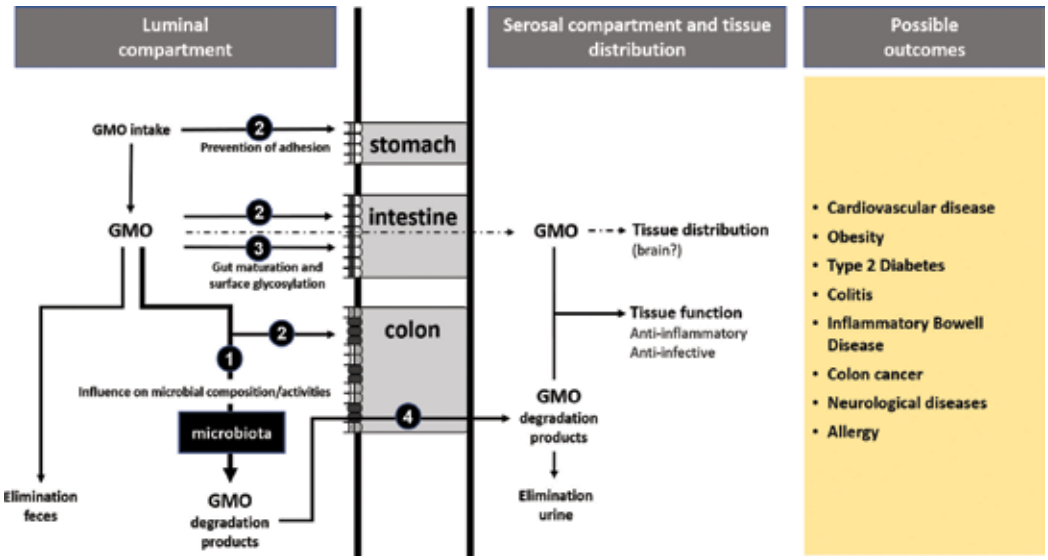

Figure 2. Metabolism of goat milk and milk derivates oligosaccharides and potential functions (1—influence on the microbiota composition and/or activity; 2—prevention of pathogens adhesion; 3—direct effects on epithelial cells; 4—systemic effects) (adapted from Kunz et al. [172]).

short-chain fatty acids such as lactic and propionic acids. However, there was no inhibition of *S. aureus* and *E. coli* grown in human feces.

The ability of oligosaccharides to reduce the pathogen binding to the intestinal mucosa is another feature that should be considered. Certain bacteria and viruses are able to recognize some types of fucosylated and sialylated oligosaccharides (which are present in milk) and adhere to them [178] reducing their adhesion to intestinal cells and consequently the occurrence of infection. Acidic oligosaccharides containing sialic acid are able to block adhesion of *H. pylori* [179], *S. aureus*, and *Clostridium botulinum* [180]. More recently, Thum et al. [181] investigated catabolism and fermentation of caprine milk oligosaccharides by selected bifidobacteria isolated from four breastfed infants. Results show that dietary consumption of caprine milk oligosaccharides (sialyloligosaccharides) may stimulate the growth and metabolism of intestinal *Bifidobacteria* spp., including *Bifidobacterium bifidum*, typically found in the large intestine of breastfed infants.

6.3.2. Anti-inflammatory activity

Martinez-Ferez et al. [182] suggested that goat milk oligosaccharides may have an anti-inflammatory action. Their research aimed to investigate whether goat's milk oligosaccharides could inhibit the adhesion of monocytes to human umbilical vein endothelial cells. The results of this research indicated that goat's milk oligosaccharides may in fact act as anti-inflammatory agents in the newborn infant, an effect that had already been demonstrated for human milk oligosaccharides and that can be attributed to the structural similarities between goat and human milk oligosaccharides.

6.3.3. Prevention of inflammatory bowel disease (IBD) and colitis

The studies of Lara-Villoslada et al. [183] and Daddaoua et al. [184] have evaluated the effects of oligosaccharides isolated from goat milk in rat models of induced colitis. Lara-Villoslada et al. [183] found that goat milk oligosaccharides are actively involved in the repairing process after a DSS-induced colitis. Moreover, the development of the intestinal flora can be stimulated by oligosaccharides containing N-acetylglucosamine that enhance the growth of *B. bifidum* [185]. Daddaoua et al. [184] reported that animals previously treated with goat milk oligosaccharides showed decreased colonic inflammation and fewer necrotic lesions compared to the respective controls. The authors suggested that the observed upregulation of the trefoil factor 3, which is involved in tissue repair, could indicate a possible mechanism of action. Although further research is needed in order to validate this approach, the use of goat milk oligosaccharides as part of a therapeutic strategy against inflammatory bowel disease seems promising.

Research data suggests that goat milk may be a very appealing source of human-like oligosaccharides The high amount of oligosaccharides in goat milk, as well as their structural profile, as opposed to other domestic mammals, place goat's milk oligosaccharides as the best source for animal-derived oligosaccharides. However, there is still a lack of data concerning the variation of the oligosaccharides profile of goat milk depending on the season, diet, lactation stage, breed, and number of lactation.

The beneficial effects of goat milk oligosaccharides are summarized in **Figure 3**. The increasing interest in healthy diets is stimulating development of new products in the food industry, and many studies have been conducted on fermented milk [138]. Taking into account the health benefits from goat milk, this could be a future trend in the field of probiotic fermented milk products. A lack of information still exists regarding traditional goat cheese and its health properties. However, the incorporation of selected lactic acid bacteria in simple and mixed cultures in goat cheese production is an example of recent innovation in this field. Furthermore, caprine milk whey could be an important source of bioactive compounds for the dietary supplement industry.

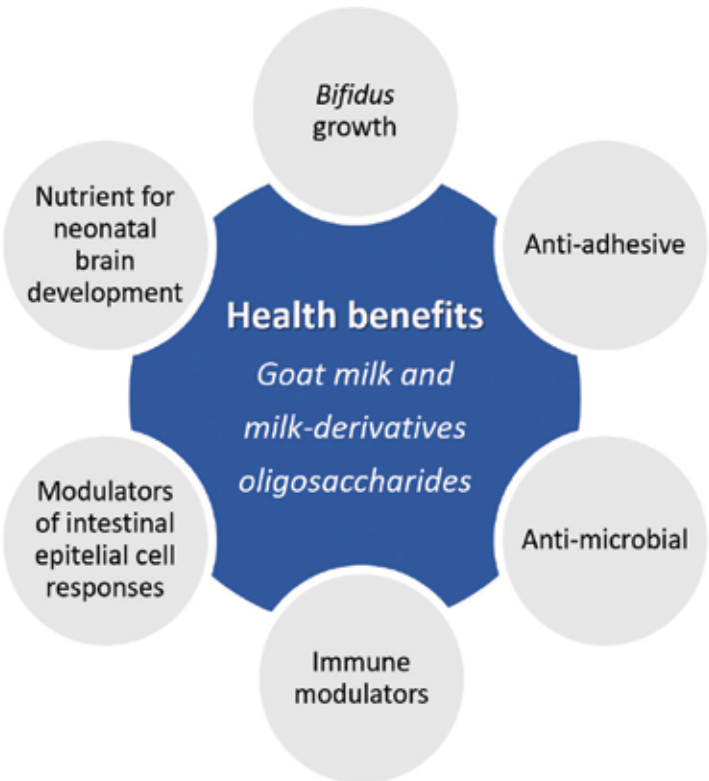

Figure 3. Main health benefits of oligosaccharides present in goat milk and its derivatives.

7. Conclusions and future prospects

Goat milk and other goat-derived products present unique characteristics and their nutritional value, as well as their potential health effects, have been the object of a fair amount of investigation. The composition of goat milk does not differ remarkably from that of cow milk, while presenting more similarities with human milk, making it more easily tolerated. Moreover, the superior digestibility of goat milk, its fatty acid composition, and the presence of various

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bioactive compounds in its constitution make goat milk potentially helpful in the treatment or even the prevention of certain medical conditions.

Several studies conducted on animal models have shown that goat milk can have beneficial effects on inflammatory bowel disease and disorders characterized by malabsorption. Goat milk, its whey, and fermented goat products may reduce the risk of chronic disorders by anti-inflammatory and anti-oxidative effects. Additionally, goat milk and its derivatives can encourage the selective growth of bacteria that are part of the intestinal microbiota, with potential benefits on the metabolic, endocrine, and immune systems. All these mechanisms suggest that the bioactive compounds present in goat's products might have pleotropic effects. The main health effects of goat-derived products are summarized in **Figure 4**.

Taking all of this into account, goat's milk and other goat derivatives have the potential to act as health-promoting food and to improve overall therapeutic success in the management of chronic diseases, in conjunction with conventional medical treatment. Goat milk is particularly recommended for infants, the elderly, and convalescing people. Therefore, goat milk and dairy products offer exciting opportunities in the area of functional foods. However, this field of research is only in its infancy, as more and more nutrients with physiological effects are being discovered. More studies and clinical trials are necessary, exploring various new fields of study, going as far as to the molecular level, in order to confirm the beneficial effects of such compounds. This will allow for the identification of important pathways in the cells and tissues of the organism and the discovery of new and accessible biomarkers that are indicative of the health benefits promoted by functional foods and their bioactive compounds.

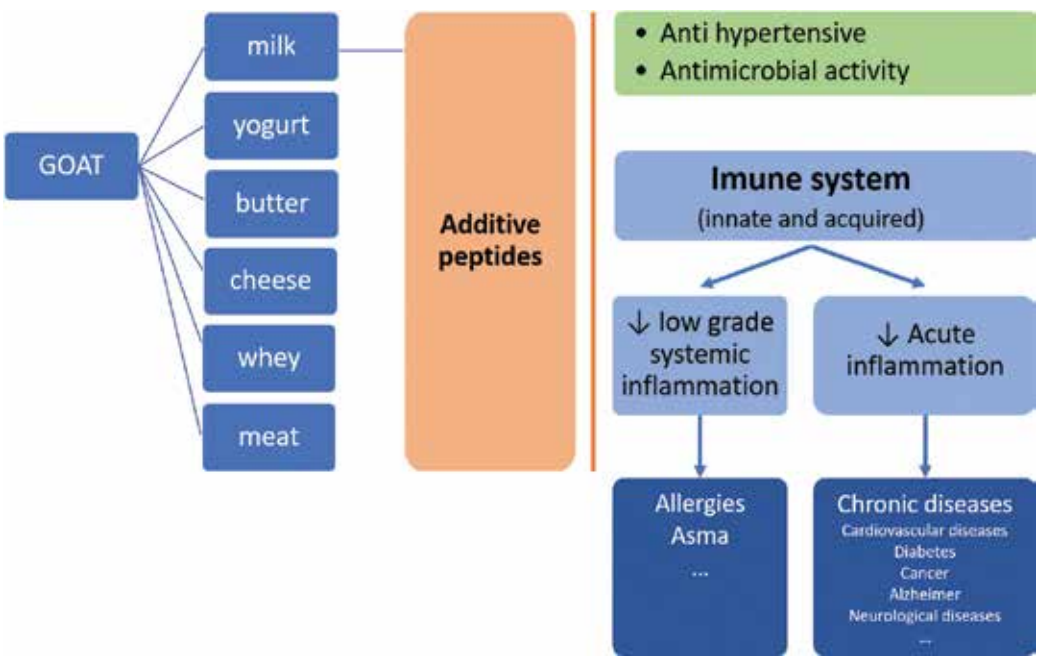

Figure 4. Schematic representation of the main health effects of goat's products.

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Chapter 11

Bioactive Compounds in Goat Milk and Cheese: The Role of Feeding System and Breed

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Abstract

This chapter provides an introductory overview of some bioactive compounds in goat milk, presenting a selection of key results from literature. The aim of the chapter is to review the effects of the feeding system and of the breed on goat milk and cheese fine quality in order to identify management options aimed at improving the nutraceutical characteristics of milk and dairy products. We will discuss a series of case studies focused on the assessment of the effects of feeding system and breed and their interaction on specific health-promoting bioactive compounds: (i) fatty acid (FA) profile, (ii) antioxidant compounds and (iii) oligosaccharides (OS). Experimental data will be discussed highlighting the potential role of local Mediterranean breeds for the production of functional dairy products.

Keywords: bioactive compounds, feeding system, goat, Mediterranean breeds, fatty acids, antioxidants, oligosaccharides, milk, cheese

1. Introduction: overview on the main bioactive compounds of goat milk

Bioactive compound, according to the National Cancer Institute (USA), is "one type of chemical food in small amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). Bioactive compounds have actions in the body that may promote good health. They are being studied in the prevention of cancer, heart disease, and other diseases". By [1], a bioactive compound is "a compound which has the capability and the ability to interact with one or more component(s) of the living tissue by presenting a wide range of probable effects". The origin of these substances can be natural—terrestrial or aquatic, a plant, animal or other

source (e.g., microorganisms)—or synthetic. The term "bioactive compound" is not attributed to the nutrient contained in food or, more broadly, to the nutrients that are essential for a living organism, such as primary metabolites.

Controversies over except the essential elements of the definition of bioactive compounds arise for food (or source of nutrition in general), where food constituents include water, carbohydrate, proteins, lipids and fatty acids, fibres, vitamins, minerals and oligo-elements. Ref. [2] consider that bioactive peptides, many vitamins, fatty acids, flavonoids and phytosterol and the soluble and insoluble fibres are bioactive compounds. Examples of bioactive compound include lycopene, resveratrol, lignan, tannins and indoles.

In recent years, functional foods and bioactive components in foods have drawn a lot of attention and interest among food scientists, nutritionists, health professionals and general consumers. A functional food may be similar in appearance to a conventional food; it is consumed as a part of normal diet but has various physiological benefits and can reduce the risk of chronic diseases beyond basic nutritional functions.

Goat milk (GM) when compared to cow milk in terms of fatty acid (FA) profile shows a larger content of medium-chain fatty acids (MCFA) such as caproic (C6:0), caprylic (C8:0) and capric (C10:0), which can be considered bioactive compounds [3]. These three fatty acids that alone represent up to 15–18% of total FA in goat milk and not more than 9% in cow milk, due to their great energy giving facility, play a key dietary role in improving lipid metabolism, especially in patients suffering from various forms of malabsorption syndromes, typically developed after intestine resection, in rehabilitating premature and undernourished infants [4]. Dietary GM improves iron bioavailability favouring the recovery of haematological parameters [5]. GM contributes to restore bone demineralisation associated to anaemia by increasing the digestive and metabolic utilisation of calcium and phosphorus. Its consumption has beneficial effects on nutritive utilisation of iron and copper [6].

The role of polyunsaturated fatty acid (PUFA), and in particular conjugated linoleic acid (CLA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA), has received much attention of nutritionists in the last 10 years. The n-3 fatty acids (i) reduce total cholesterol and low-density lipoprotein cholesterol (LDL) levels but increase high-density lipoprotein (HDL) cholesterol, (ii) counteract hypertension, (iii) play a role in the regulation of hormonal secretion and (iv) are beneficial in the care of skin pathologies and are also useful in the therapy of arthritis and other inflammatory problems.

The acronym CLA is used to express the mixture of isomers of the linoleic fatty acid with double conjugated bonds, located, above all, on the atoms of carbons 9 and 11. Biological activity is mainly attributed to rumenic acid (C18:2 cis 9, trans 11), which represent about 90% of the total isomers present in the fat of ruminants [7]. The CLA in milk has two origins: from the rumen biohydrogenation of unsaturated fatty acids, present in substantial quantities in fresh forage, and from the synthesis in animal tissue, mainly the mammary gland and adipose tissue, starting with the vaccenic acids (VA) through the action of the delta 9-desaturase enzyme.

The amount of these biologically active molecules in milk and cheese fat from ruminants is affected by animal diets [8]. There have been studies about grazing based on shrub and woody lands affecting CLA and VA content in milk and cheese from sheep and goats [9] also with regard to specific forage species in the pasture [10]. Nevertheless, our knowledge on the effects of common Mediterranean forage species, agronomic management, forage conservation and breed on goat milk bioactive fractions is still limited.

Recently, another topic of interest has been the antioxidant content in milk and cheeses. In milk there are several antioxidant compounds which can be classified as enzymatic antioxidant and non-enzymatic antioxidant. Among antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidases have been demonstrated in milk. Non-enzymatic antioxidants, lactoferrin, vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids and polyphenols can be formed in the animal body or need to be supplied in the feed as essential nutrients [11, 12]. Several non-enzymatic antioxidants act as radical scavengers in the lipid phase, such as vitamin E, carotenoids and ubiquinol, whereas vitamin C acts in the water phase. Others can react in both the lipid and the water phase, such as some polyphenols (flavonoids), which operate both as radical scavengers and metal ion binders [12]. The parameters that are taken more into consideration are the beta-carotene and α-tocopherol content and the level of protective antioxidants. A molecule is recognised as an antioxidant when it is able to slow down, or hinder, oxidising processes against certain substances. A synthetic index of this capability is represented by the degree of antioxidant protection (DAP) [13]. The DAP is calculated as ratio between the amount antioxidant element (e.g., α-tocopherol) and the element to be protected against oxidation (cholesterol).

Increasing α-tocopherol in milk is important not only to enhance its nutritive value but also to prevent lipid oxidation which leads to rancidity of milk and dairy products; vitamin E supplementation is a standard practice in most farming systems. Milk tocopherol content depends on several factors such as breed, feed and stage of lactation; large differences exist among ruminant species and within species.

Very little is known on the effect of diet on the content of non-volatile phenolic compounds in milk or cheese. The results of a few recent studies demonstrate the accumulation of various phenolic compounds in the milk of grazing goats [14, 15]. High content of phenols in milk has shown to improve the quality of milk, such as its oxidative stability of the process' efficiency and quality of dairy products [16].

The interest towards drinking goat milk is increasing, due to the recognised nutritional properties of this milk in comparison with cow milk [17, 18]. A class of bioactive compounds recently rose to major interest, namely, the oligosaccharides [19], due to their beneficial effects on human health as intestinal inflammation [20] and on brain development and immunity in infants [21]. The content of goat oligosaccharides (OS) compared to other domestic ruminants milk is about 4–5 times higher than cow milk and up 10 times than sheep milk [22]. The scarce availability of those from human milk encouraged to deepen studies on these bio-compounds. The studies showed OS content and profile in goat milk most similar to breast milk in comparison to other farm mammalians, in particular as far as fucosylated and sialylated OS to human milk oligosaccharides [23], as to suggest, by several authors, the use in the production of products for human nutrition, such as infant formulae.

This chapter provides an overview of the main bioactive compounds in milk and goat cheese (fatty acids, antioxidant and oligosaccharides) conveying data from significant case studies carried out at the experimental farm of Council for Agricultural Research-Unit for Extensive Husbandry (CREA-ZOE), located in Bella (Muro Lucano, Potenza), Basilicata region (Southern Italy).

2. Feeding strategies affecting the bioactive compounds in milk and cheese

2.1. Fatty acids affected by feeding regimen

Nutrition is a natural and low-cost way for farmers to rapidly and sharply modulate milk and cheese FA profile towards a healthy profile [24]. The composition of milk fat reflects to some extent the composition of the dietary fat, despite the hydrogenation and isomerisation process to which the FA may be subjected in the rumen. Forages, even though containing a relatively low level of lipids, are often the major source of beneficial unsaturated fatty acids in ruminant diets, and they also provide a low-cost approach to improve milk FA profile in comparison with diet supplementation strategies. In literature, several studies have focused on the impact of different diets on the main milk FA classes, and they also have examined the associations between feeding of various forages and FA composition of milk fat.

Among forages, legumes deserve a special attention due to the raising number of farmers in conversion to organic and low-input production system (i.e. the environmental role of legumes in cropping systems has been even enhanced in Europe by common agricultural policy (CAP) reform) but also to the need of reducing the dependence on the import of protein-rich feed material. Even if, for a given crop, substantial within-species variation occurs, altogether some legume forages such as white clover and birdsfoot trefoil can be considered a rich source of PUFA [25]. Birdsfoot trefoil PUFA content (19.4 g/kg DM) was found higher than in many other legumes, grasses and forbs [26], while white clover with an average ALA content of 16 mg/g DM was a richer source than other common forage legumes (alfalfa, trefoil and red clover) and grasses (orchard grass, fescue and timothy) [27]. Fresh grass is the one main source of ALA. It has been recognised that favourable changes in milk FA profile can be obtained by grazing or feeding fresh forages. Several studies have shown that milk from grazing goats is naturally enriched in fatty acids considered as favourable for human health in comparison to goats fed with high-concentrate diets [24, 28].

Goats unlike sheep are predominantly browsers; in Mediterranean shrublands browse can account for up to 60–80% of goat's diet; animals well adapted to tannin-rich woody forage sources can consume relatively large amounts of tannins without suffering any systemic toxicity [29]. While tannin content in forages is negatively correlated with voluntary intake, digestibility and nitrogen retention, a relatively low amount in ruminant diet can positively affect milk FA composition by protecting dietary PUFA against rumen biohydrogenation [30]. Many forage legumes such as clovers, vetches and Sulla (*Hedysarum coronarium*) are a rich source of polyphenols and especially tannin phenols (TP). Ref. [31] observed that condensed tannins (CT) in Sulla

at flowering were contributing to a higher concentration of linoleic and ALA in ewe's milk and a lower ω6/ω3 ratio. Refs. [32, 33] showed that red clover silage, which contained levels of ALA similar to that of grass silage, improved milk PUFA due to a high proportion of red clover ALA passing through the rumen. Polyphenol oxidase (PPO), which is the enzyme involved in the browning reaction of red clover leaves when cut or crushed and exposed to air, has been found to reduce protein and lipid degradations in vitro and potentially in the rumen [34].

Overall, this brief overview on the role of feeding regimen in modulating goat milk fatty acid profile shows that both farm-grown forages including legumes, as well as native pastures, can be considered an effective low-cost way to improve goat milk FA composition without compromising yield and opening new alleys towards a sustainable intensification of the extensive dairy goat system.

2.1.1. Case study: feeding and season on milk fatty acid profile

In this section we report a study carried out at the experimental farm of Council for Agricultural Research and Economics-Research Unit for the Extensive Animal Husbandry (CREA-ZOE) located in Bella (Basilicata region, Southern Italy) during winter, spring and summer seasons. In order to examine changes in milk FA profile under the effect of different feeding regimes, typical of Mediterranean extensive and semi-extensive goat production systems, four groups of Mediterranean Red goats were formed and allocated to different feeding systems: (G) grazing on a native pasture (8 h/day) without supplementation, (GS1) grazing supplemented with 550 g/d of maize and broad beans (CP 14% and NDF 18%, slowly degradable), (GS2) grazing supplemented with 550 g/d of barley and chickpeas (CP 14% and NDF 18%, rapidly degradable) and (H) housing and fed with hay produced with the grass from the same pasture plus 550 g/d of mixed grains (CP 15% and NDF 18%) [35–38]. Regarding lipid extraction method, briefly milk sample (10 ml) was homogenised (2 min) with $CHCl_3$ and MeOH mixture (2/1, v/v) and centrifuged (500 × g, 10 min). After removing the upper layer, the lower layer was filtered through a Buchner funnel, rinsed with $CHCl_3$ (30 ml) and then again filtered. The chloroform-lipid extract was dried over anhydrous Na_2SO_4, rinsed with $CHCl_3$ (30 ml) and concentrated using a rotary evaporator at 30°C. The residue was stored at −80°C for lipid determination. Lipid extract was methylated adding hexane (1 ml) and 2 N methanolic KOH (0.05 ml). Gas chromatograph analysis was performed on a Varian model 3800 GC instrument fitted with an automatic sampler (CP 8410) for a multiple injection. Fatty acid methyl esters (FAME) were separated through a cyanopropyl polysiloxane (DB 23, J & W) fused silica capillary column (60 m × 0.25 mm i.d.). Operating conditions were a helium flow rate of 1.2 ml/min, a FID detector at 250°C and a split-splitless injector at 230°C with a split ratio 1:100. The column temperature was held at 60°C for 5 min after sample injection (1 μl), increased at 14°C/min to 165°C and at 2°C/min to 225°C and held at 225°C for 20 min. The individual fatty acid peaks were identified with reference to the retention times of standard of CLA isomers (cis-9, trans-11 97% and trans-10, cis-12 3%; Larodan, Malmö, Sweden) and a known mixture of standards (FAME, Sigma). Fatty acids were expressed as percentage of total FAME.

Milk produced by goat groups showed a wide variability in its FA composition linked to the characteristics of the ingested feed in each type of feeding system (**Figure 1**). In particular,

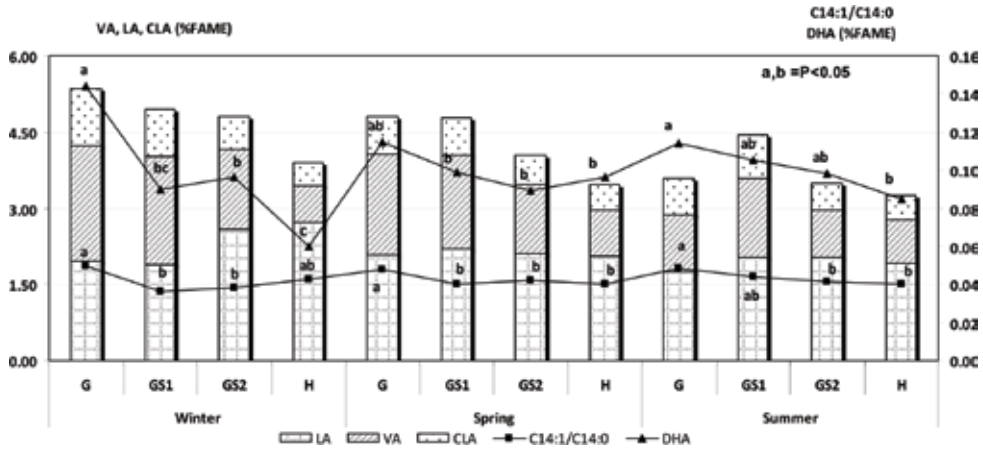

Figure 1. Effect of feeding system × season interaction on vaccenic acid (VA), linoleic acid (LA), conjugated linoleic acid (CLA) and docosahexaenoic acid (DHA) content and on Δ9-desaturase activity (C14:1/C14:0) of milk of goats fed with (G) grazing on native pasture without supplementation, (GS1) grazing plus maize and broad beans (GS2) and grazing plus barley and chickpeas and (H) housed and fed with hay plus mixed grains (modified from Refs. [35–37]). a, b and c = $P < 0.05$.

G and GS1 groups produced milk with a higher content of CLA and VA compared to other groups. Indeed, it is noted that the consumption of high-concentrate diets, compared with high-forage ones, affects the extent of ruminal biohydrogenation with a consequent reduction of CLA and VA production. The high variability of CLA and VA levels in milk of G group, with the highest level reached in winter, could be ascribed to the seasonal changes in grass availability and in phenological stage of the plants. The similar pattern of VA and CLA observed in milk of goat rearing in different feeding systems confirms the positive relationship between these intermediate products of ruminal biohydrogenation. Feeding regimen and season also affected Δ^9-desaturase activity (C14:1/C14:0), responsible of endogenous synthesis of CLA, with fresh grass being able to enhance this enzyme activity (**Figure 1**).

Grazing significantly increased the proportion of long-chain n-3 PUFA, such as DHA, and decreased the n-6/n-3 PUFA ratio in milk (**Figure 2**). The level of DHA reached interesting value in milk fat of grazing goats in winter probably because of the high content of its precursor (ALA) in the pasture. The ratio between n-3 PUFA and n-6 PUFA is an index commonly used to assess the nutritional value of fats [39]. Housing goats exhibited a higher n-6/n-3 PUFA ratio than other treatments, probably attributable to the high level of LA in milk (**Figure 1**), the main component of n-6 PUFA. The composition of concentrate mixture offered to H group appears to explain the highest content of LA found in milk fat.

The distribution of concentrates to grazing goats significantly affected milk FA profile. Under grazing condition, GS1 dietary treatment characterised by slowly degradable concentrate improved milk FA profile compared to GS2 group fed with rapidly degradable concentrate. Probably the supplementation received by GS1 group could have determined a rumen environment favourable to a less efficient biohydrogenation of substrate with consequent accumulation of intermediate products. Besides, the differences observed in milk FA composition between supplemented and non-supplemented grazing groups could be linked to the different herbage selections of supplemented grazing goats, as suggested in Ref. [40].

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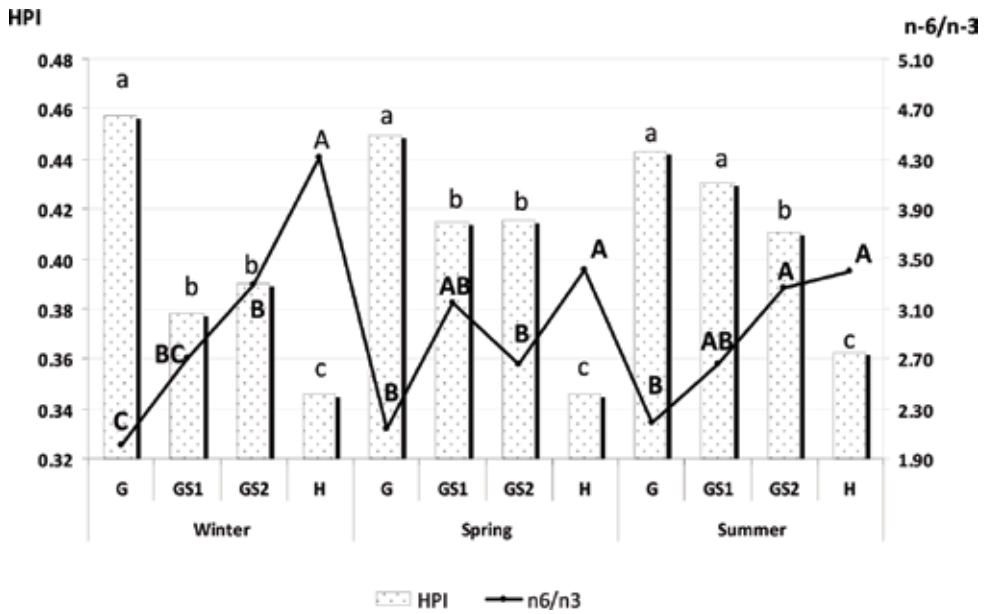

Figure 2. Effect of feeding system × season interaction on n-6/n-3 and health-promoting index (HPI) value of milk of goats fed with (G) grazing on native pasture without supplementation, (GS1) grazing plus maize and broad beans and (GS2) grazing plus barley and chickpeas and (H) housed and fed with hay plus mixed grains [modified from Ref. [38]). A, B and C = $P < 0.01$ for n6/n3. a, b and c = $P < 0.05$ for HPI.

The effect of different feeding systems on beneficial FA in milk is more evident using the health-promoting index (HPI, **Figure 2**), an index that expresses the health value of dietary fat, and it is calculated as follows: total unsaturated FA/[C12:0 + (4 × C14:0) + C16:0] [41]. Dairy product with high HPI value is assumed to be more beneficial to human health. According to this index, pasture feeding allows the optimisation of the balance between detrimental and valuable fatty acids in goat milk, thus obtaining beneficial effects for consumer's health.

The results of this study show that milk from goats fed with pasture had higher amounts of nutritionally peculiar FA than milk from other feeding treatments. On the other hand, grazing supplementation with concentrates that better interact with the nutritive characteristics of pasture could represent a strategy to meet nutritional requirements of animals and sustain milk production without worsening its quality.

2.1.2. Case study: relationship between forage species and fatty acids of cheese

In this section we report a study carried out at the CREA-ZOE experimental farm in spring. Eight homogeneous groups of Red Syrian goats have been allocated to eight different feeding treatments. The housed goat groups received during 11 days a single forage species *ad libitum*, and they had access to water and salt blocks. Daily, the forages were cut and provided to the goat groups. Seven forages (*Avena sativa*, *Lolium perenne*, *Hordeum vulgare*, *Lotus corniculatus*, *Medicago sativa*, *Trifolium incarnatum*, *Vicia sativa*) were used at the phenological stage commonly used in Southern Italy for grazing and one (*Triticosecale*) as silage. After an adaptation period to the forage

supplied, the milk of each group was collected and processed into *Caciotta* cheese, a traditional goat cheese manufactured in Southern Italy, ripened for 20 days. Cheese samples (3 g) were finely grated, and lipid extraction and composition were performed as described in Section 2.1.1.

In this study, forage species had an effect on FA profile of *Caciotta* cheese [42]. As regards CLA (**Figure 3**), cheeses from goat groups fed with *T. incarnatum*, *Triticosecale* and *H. vulgare* showed higher values than those obtained from *A. sativa* and *L. corniculatus*. The lowest content of CLA and the highest content of ALA were detected in the cheese made from milk of goats fed with *V. sativa*. Cheeses from *L. corniculatus* and *M. sativa* displayed the same ALA content. The ALA showed a major variation among cheeses (range 1.04), while the CLA exhibited a smaller variation (range 0.349) [42–44].

Cheeses from legume groups showed significantly higher values of ALA compared with grass groups, whereas the content of ALA in cheese from *Triticosecale* silage was in trend with other fresh grasses. Green fodders are excellent source of ALA and are the most effective feeds in shifting the milk FA profile towards a healthy profile. Fortunately, the milk processing does not change substantially its FA profile [45]; it follows that the bioactive compounds, CLA and ALA, of dairy products are dependent of their content in the unprocessed raw milk [43, 46]. The effects of different forage species on ALA, VA and CLA content in cheeses could be connected to the high content of PUFA in green forage, with ALA being the most representative of this FA class (**Figure 4**), and to the role of secondary metabolites (polyphenols) and vegetable enzymes (polyphenol oxidase) present in forage species. These compounds have potential to interact with lipolysis and biohydrogenation of PUFA in vitro, in fact a negative relationship was found between tannic polyphenols/ALA content ratio and ALA biohydrogenation [47]. In our study, the highest content of ALA in cheese from goats fed with *V. sativa* could be linked to the higher level of tannic polyphenols in the forage as reported by Ref. [48].

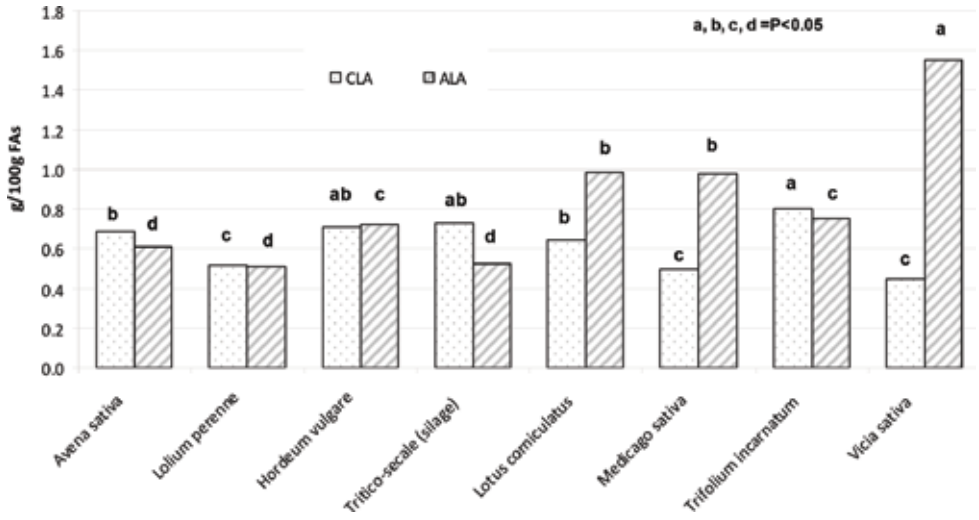

Figure 3. Comparison of conjugated linoleic acid (CLA) and α-linolenic acid (ALA) of Caciotta cheese made from milk of goats fed *ad libitum* with a single forage species (modified from Refs. [42–44]). a–d = $P < 0.05$.

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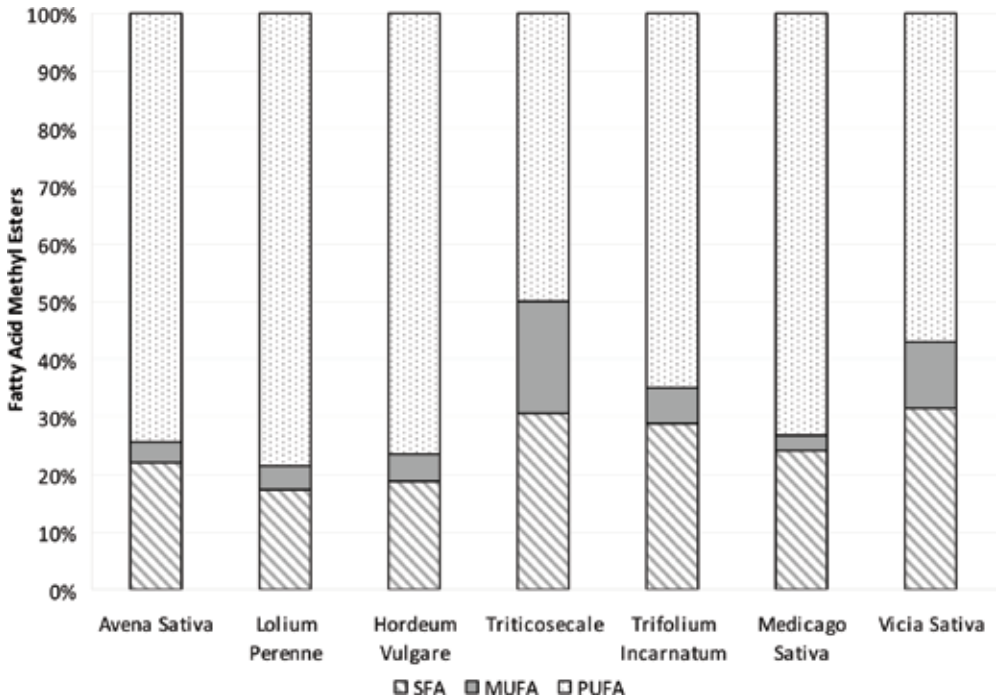

Figure 4. Comparison of percentage content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in fresh forage species (authors' own unpublished data).

The health-promoting index was calculated in order to have an immediate view of the bioactive compounds present in the cheese [41]. The forage species affected the HPI (**Figure 5**). The higher HPI values observed in *Caciotta* cheese from goat groups fed *H. vulgare* and *A. sativa* [43, 44] could be linked to the high level of PUFA found in these forages (**Figure 4**). The HPI observed in other cheeses is still higher compared to those found in milk from animals fed with dry fodder (see Section 2.1.1).

This case study shows that the single forage characterises the bioactive compounds' content in cheese; this result can be a strategy to guide, depending on farm fodder availability, the production of dairy products beneficial to human health.

2.2. Antioxidant compounds in goat milk

Measuring the total antioxidant capacity of milk and cheese helps to understand the relationships between the bioactive compounds present in milk and their ability to protect the substrate. Antioxidant activity can be enhanced by providing food as a source of antioxidant components [16]. The results of a few recent studies show the accumulation of various bioactive compounds biotransformed and/or as such in the milk and cheese of grazing goats or fed with a mixture of forage legume [18, 49]. The high value of total phenolic concentration with added nutritional and sensory values, without changing properties of the cheeses itself, was observed in cheeses made from goats fed with non-distilled thyme leaves, one of the aromatic

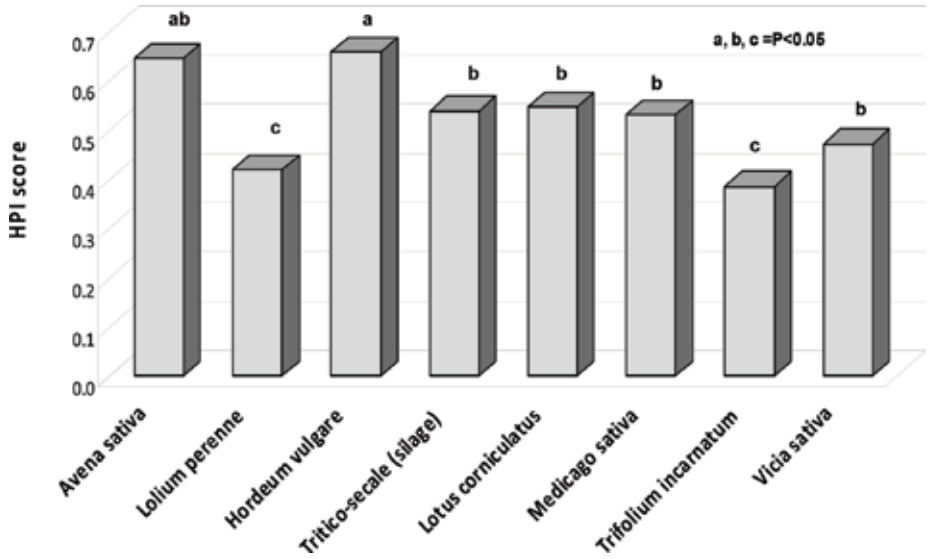

Figure 5. Comparison of health-promoting index (HPI) of *Caciotta* cheese made from milk of goats fed *ad libitum* with a single forage species (modified from Refs. [43, 44]).

plants widespread in the Mediterranean area [50]. The influence of high-polyphenol diet on cheese total polyphenol content and antioxidant capacity has been reported by Ref. [51]. They found higher level of polyphenolic compounds and antioxidant activity in cheese produced with milk from grazing goats, with a rich content of secondary metabolites, in comparison with cheese from goats kept in full indoor confinement and fed with Lucerne hay and concentrate. Grazing management represents a better option than indoor feeding to produce a healthy profile of bioactive compounds, providing an increase of total polyphenol, hydroxycinnamic acids and flavonoid concentrations. The feeding strategy involves not only polyphenols but also fat-soluble vitamins, especially those that play an important role as antioxidant (α-tocopherol and β-carotene). A positive relationship was observed between pasture-based rations rather than the hay-based rations for goats and levels of α-tocopherol and retinol in Rocamadour cheese, while β-carotene was not detected [52]. Ref. [49] found that grazing level high and medium, as percentage of net energy of requirement recovered from pasture, on Mediterranean shrublands and month of grazing also affect α-tocopherol content in goat milk without change of milk total antioxidant capacity.

Among forages, legumes are a rich source of polyphenols; large variability occurs among species; some ancient crops like common vetch (*V. sativa*) were found to contain threefold more polyphenol and five times more flavonoids than soybean [53]. Another important class of antioxidant compounds in legumes is represented by carotenoids and tocopherols; as many other compounds, their concentration is influenced by leaf proportion. Ref. [54] recommend a strategic approach to the choice of harvest date and wilting duration, since these can be a key tool for manipulating vitamins and FA composition in forages. Among forage legumes birdsfoot trefoil shows a relatively high content of lutein and contains almost threefold higher concentration of α-tocopherol content than yellow sweet clover and Lucerne (65 vs. 23 and

21 mg/kg dry matter, respectively) [26]. In forages, however, the complex mechanisms of interaction between pro- and antioxidant compounds must be taken into account [55]. While legumes might not be the richest/most effective source of α-tocopherol, it is the synergy between the phenolic acids, CT and anthocyanins that contribute to build up the so-called antioxidant network [56]. Altogether, the choice of natural feeding strategies for goats, without the use of expensive supplements, synthetic and/or encapsulated, could provide a way to encourage the consumer to the choice of dairy products obtained with natural resources and associated with beneficial health effects beyond its pure nutritional value.

2.2.1. Case study 1: borage and hawthorn and phenolic compounds in milk

Mediterranean pastures are highly variable in relation to the season, the proportion of grass plants decreases from 85 to 55% from winter to spring, while forbs increase from 25 to 65% from late spring to early summer. In early summer, goats graze mainly on forbs, some of which are used as medical plants by human. In order to highlight a relationship between non-volatile phenolic compounds in plant species and the same class of metabolites in milk or cheese, Ref. [14] examined the nuclear magnetic resonance (NMR) spectra of two green plants, borage (*Borago officinalis* L.) and hawthorn (*Crataegus oxyacantha* L.) (**Figure 6**) and milk (**Figure 7**) obtained from two groups of goats fed *ad libitum* with these plants during 18 days. A control group was fed *ad libitum* with natural hay and concentrate. Briefly, air-dried plants (500 g) were extracted with $CHCl_3$ and MeOH (10%, w/v) at room temperature. Chloroform extracts were fractionated on silica-gel column (80 × 4 cm) eluting with $CHCI_3$ and $CHCI_3$-MeOH mixture of increasing polarity. Fractions were purified by RP-HPLC on a μ-Bondapak column eluting with H_2O-MeOH (1:1). Methanol extracts of plants were fractionated between BuOH and H_2O to give a butanol residue, which was chromatographed on Sephadex LH-20 eluting with MeOH. Fractions were purified by RP-HPLC as reported above. Milk sample (1 L) was lyophilised and then extracted, fractionated and purified as described for plant sample. The structure of the pure compounds isolated from samples was determined by analysis of ^{1}H, ^{13}C and ^{13}C DEPT NMR data (Bruker DRX 600 NMR Spectrometer; Bruker, Karlsruhe, Germany) and by comparison with literature data. Compound identification was also confirmed, when possible, by HPLC analyses with reference to the retention times of standards (Sigma-Aldrich Co., Milan).

The authors found a relationship between the antioxidant intake from borage and hawthorn and the levels of antioxidant metabolites in milk, flavonoids and terpenoids contained in these herbs that were found in milk. Quercetin and rutin were excreted in part without modification, while other compounds were structurally modified. No metabolite has been found in the control group milk. The different solvents, methanol or chloroform, used in the complex method of extraction for the plant material and milk have generated great differences in the recovered metabolites. For the purpose of a useful comparison of results from different experiments, the standardization of extraction methods appears to be desirable.

Thus, the hypothesis of the authors was that gastrointestinal microflora of goats can structurally modify plant metabolites through hydrolyses and/or other interactions that result in structurally less complex molecules in milk. This study demonstrates that the presence of phenolic compounds in milk depends on the animal feed.

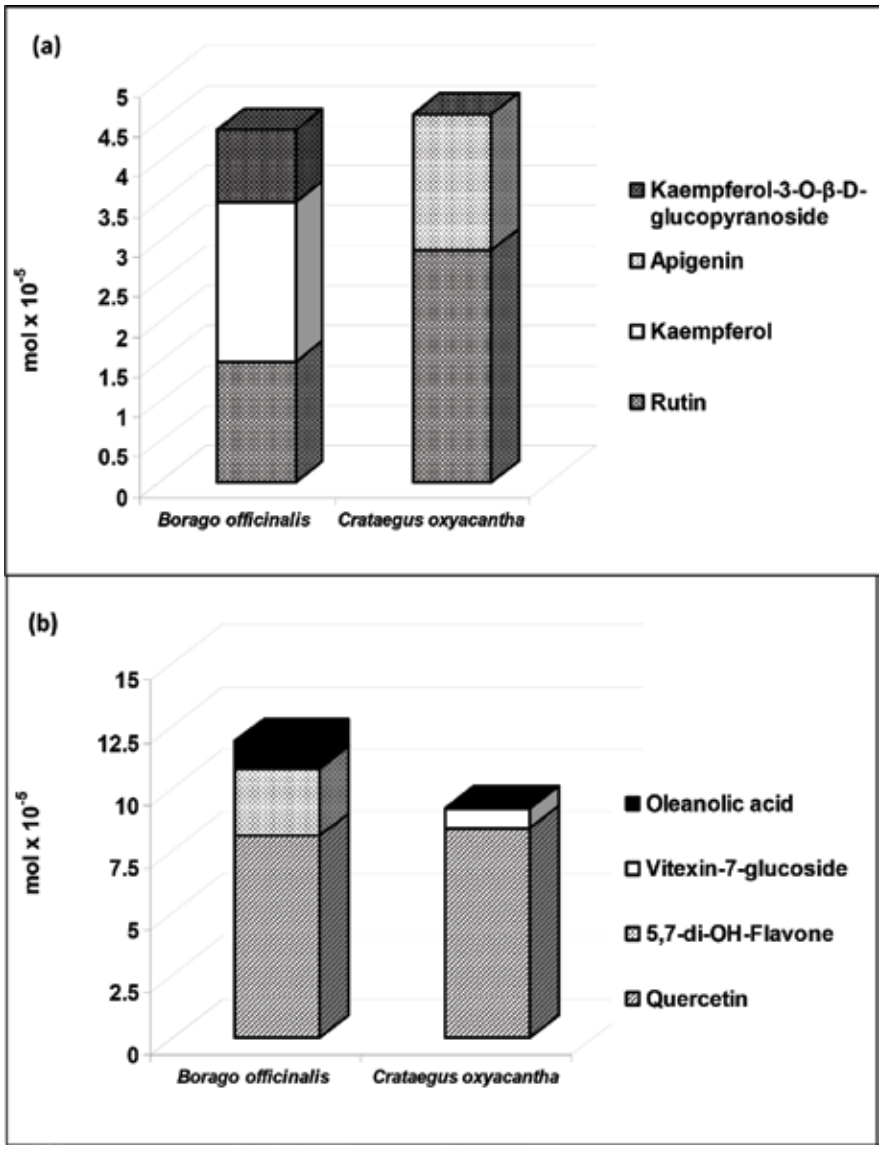

Figure 6. Metabolites found in plant of *Borago officinalis* and *Crataegus oxyacantha*. Plant extract with methanol (a) and chloroform (b) (modified from Ref. [14]).

2.2.2. Case study 2: oat and phenolic compounds

The wild species or aromatic plants in the pasture are less present in quantity than forage species. As forages represent a high proportion of ruminant diet, in order to observe the link between phenolic content of forage species and phenolic content of milk, whey and cheese, Ref. [15] planned an experiment with ten Mediterranean Red goats fed indoor with fresh *A. sativa* forage, in pureness, without any other supplementation. After 10 days of adaptation

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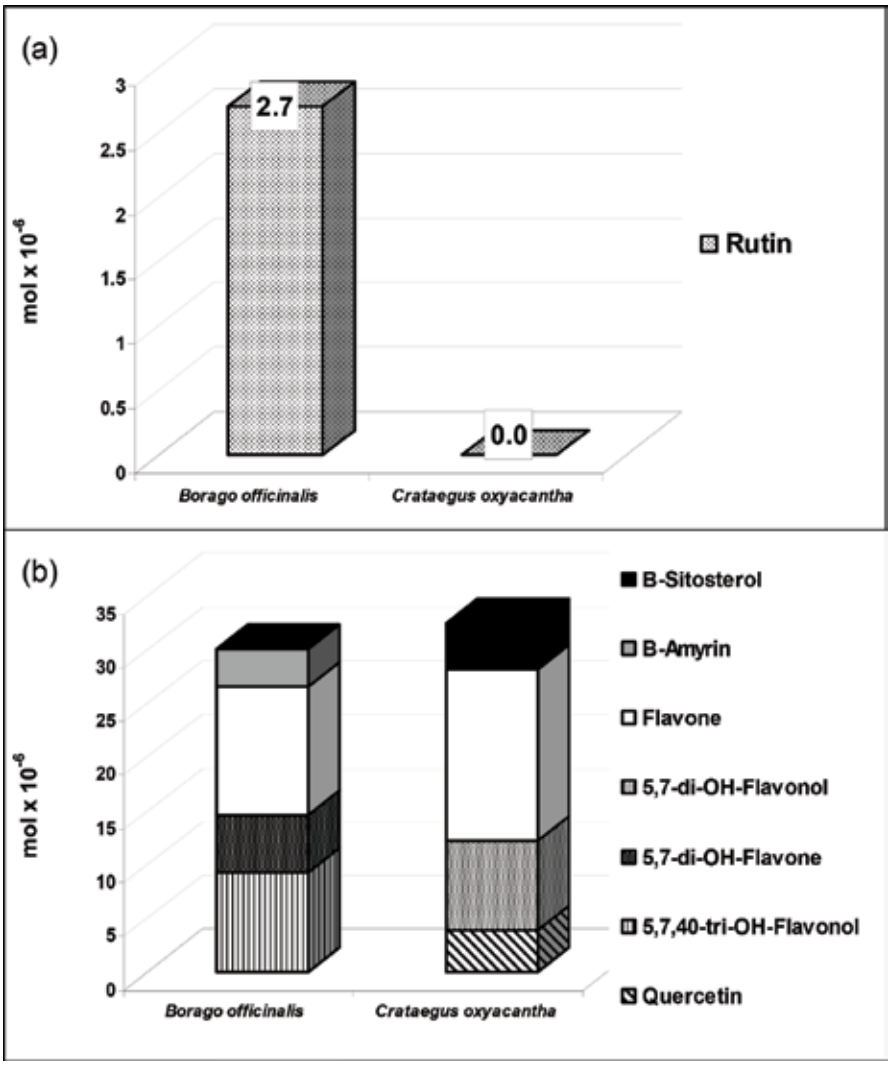

Figure 7. Plant metabolites found in milk from goats fed with *Borago officinalis* and *Crataegus oxyacantha*. Milk extract with methanol (a) and chloroform (b) (modified from Ref. [14]).

and 3 days of experiment and sample collection, phenolic compounds were extracted from herbage, milk, whey and cheeses by methods appropriate to the substrate and analysed by high-performance liquid chromatography (HPLC-DAD).

Ten Siriana goats were fed indoor with *A. sativa* in pureness, given fresh for 10 days of adaptation and 3 days of trial. In these 3 days, herbage (the part of plant effectively ingested) was daily collected, freeze-dried and ball milled. Milk and whey samples were collected on days 2 and 3, contextually with cheesemaking (*Caciotta* cheese) and stored at −20°C. Briefly, phenolic compounds were extracted from herbage (200 mg) with EtOH/H_2O (80:20) at 90°C while from milk (10 ml) and whey (10 ml) by a precipitation in CH_3CN (22 ml)

and an overnight deconjugation using a glucuronidase-sulfatase enzyme mixture. Cheese was homogenised and centrifuged, and the supernatant was used for phenolic extraction as described for milk. Phenolic compounds of the extracts were analysed as UV-absorbing compounds using HPLC-DAD on a reverse phase column (LiChroCART® 125-4, Merck) eluted by 0.3 ml/min of a 0–100% gradient of acetonitrile in water, both containing 0.1% formic acid. The UV spectra were compared to those of standard compounds and classified into simple phenol, benzoic acid derivatives, cinnamic acid derivative and flavones groups. The *A. sativa* forage revealed an interesting metabolite profile, where cinnamic acid and flavones largely occur (mostly derived from apigenin and luteolin or chrysoeriol). These phenolic compounds affected milk and whey, even though in different measures: flavones disappeared, while simple phenols, benzoic acid derivatives and some unclassified phenols were identified (**Figure 8**).

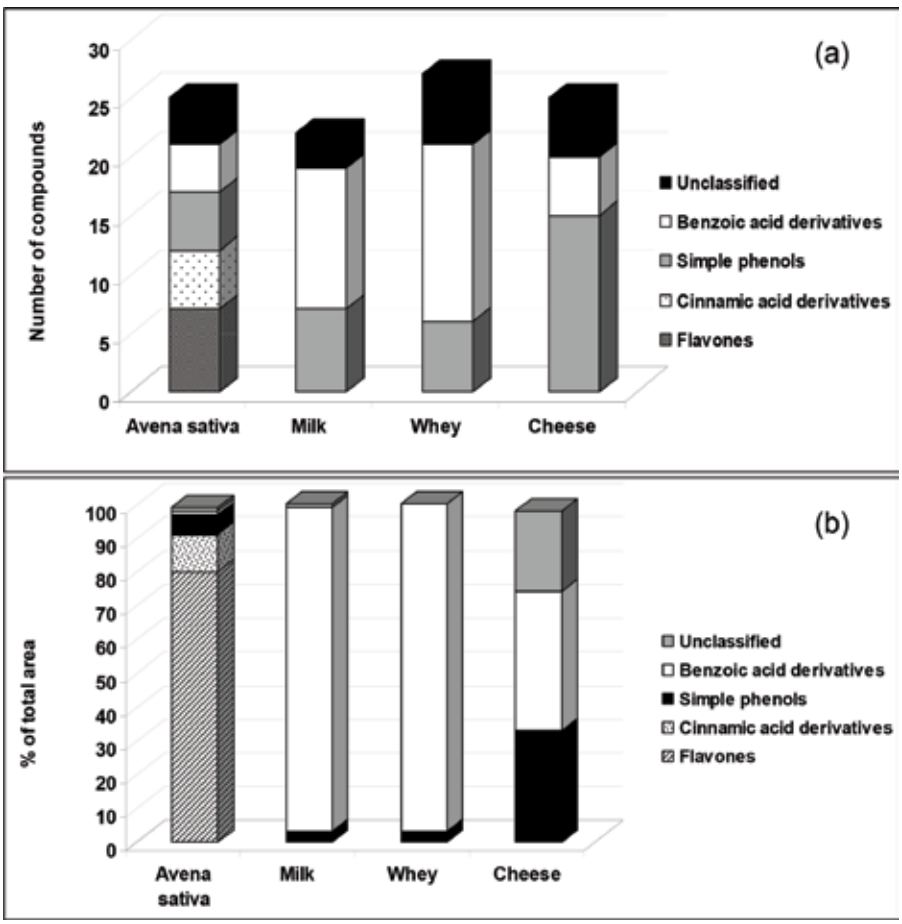

Figure 8. Phenolic compounds in *Avena sativa* forage and milk, whey and cheese from goats fed with fresh *Avena sativa* in pureness. (a) Data are expressed as abundance of compounds and (b) percentage of total picks by HPLC-DAD analyses (modified from Ref. [48]).

In cheese, although the largest amount of phenolic compounds was still by benzoic acid derivatives, there was a greater number of simple phenols and one of the indole derivatives found in milk. Nevertheless, phenolic compounds' profile of milk was much closer to whey profile than to cheese ones.

These preliminary results have allowed us to get an overview of the transfer of the plant metabolites directly or processed or degraded in the digestive tract, to the product. However, quantitative studies would be desirable to measure the partition of phenolic compounds in serum and cheese.

2.2.3. Case study 3: relationship between forage species and antioxidant compounds in milk

In goat feeding, forage plants such as grasses and legumes have an essential role, since they represent a high proportion of diet. Forages commonly used in Mediterranean area can be a natural source of bioactive compounds that can be transferred to animal products. In order to evaluate and compare the potential contribution of some grass and legume species, to increase the level of bioactive compounds and antioxidant capacity in milk, Refs. [48, 57] compared the total polyphenol intake of three grasses, *Festuca arundinacea*, *H. vulgare* and *Triticosecale*, and four legumes, *Pisum sativum*, *Trifolium alexandrinum*, *V. sativa* and *Vicia faba minor*, given to seven groups of Mediterranean Red goats without supplement for eleven days. The single forage was cut daily and given *ad libitum* indoor. After an adaptation period, forage samples and milk samples of each group were collected and analysed for polyphenolic compounds and total antioxidant capacity. Folin-Ciocalteu method as described by Ref. [60] was used to determine tannic and non-tannic polyphenol contents in forage samples, after the addition of insoluble matrix polyvinylpolypyrrolidone (PVPP) and total and free polyphenol contents in milk samples. Contents were expressed as gallic acid equivalents (GAE). Milk conjugate polyphenol content was obtained by difference between milk total and free polyphenol contents. Total antioxidant capacity (TAC) was measured using the ferric-reducing antioxidant power as indicated by Benzie and Strain method and was expressed as μM $FeSO_4$.

Among forages, *T. alexandrinum* and *F. arundinacea* were shown to enhance the milk-free polyphenol content (**Figure 9**) and the antioxidant capacity (**Figure 10**), respectively.

The phenological stage to which the fodder was used by goats may have contributed, as reported in the literature [47], to total polyphenol intake (**Figure 9**) and milk polyphenol content. As polyphenolic beneficial compounds occur largely in forages, it could be assumed their possible relationships and their transfer from diet, through some biotransformations or, as such, to milk according to De Feo et al.'s [14] study.

2.2.4. Case study 4: Sulla forage and phenolic compounds and antioxidant capacity

Among plant species that are used in ruminant feed in the Mediterranean area, Sulla (*Sulla coronarium* L.), which is a short-lived perennial legume, plays a key role in the cereal-based systems that are used in semiarid regions. This legume forage has widespread availability in Mediterranean areas, where it is greatly appreciated for the positive effects of its nutrient and CT contents on milk yield and composition, as demonstrated in both sheep and goats [58, 59].

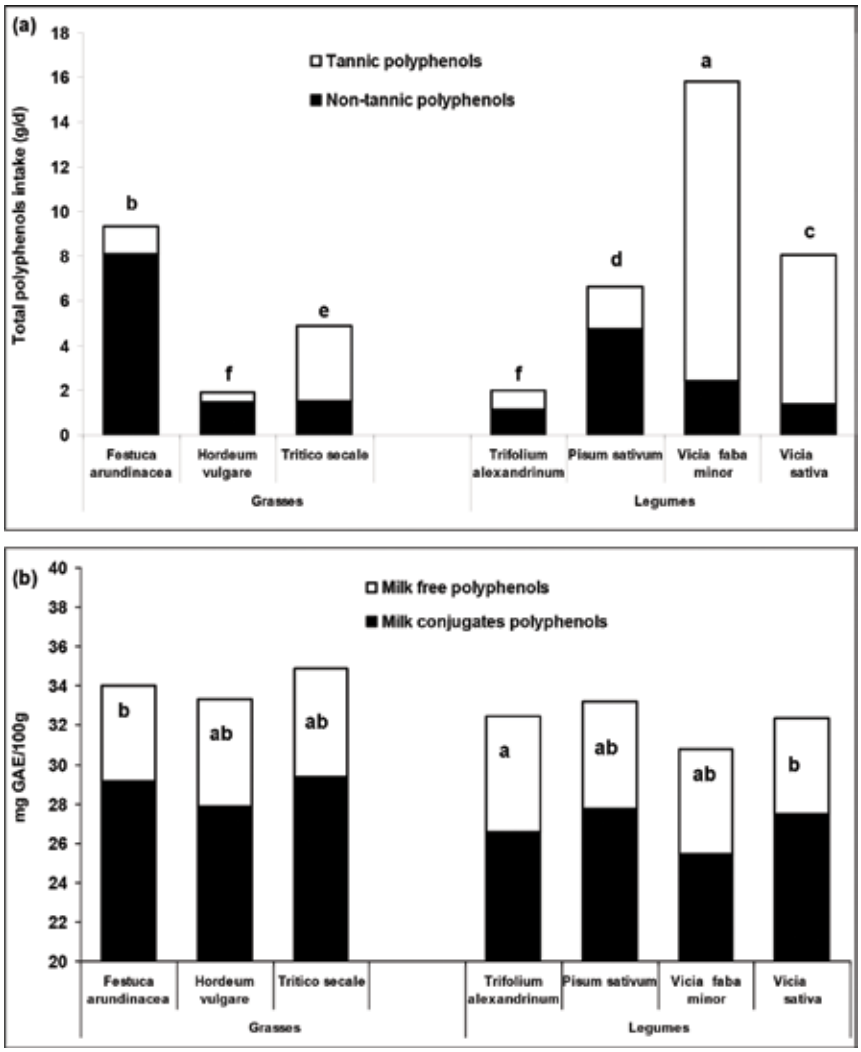

Figure 9. Comparison of total polyphenol intake (a) from goats fed *ad libitum* with a single species, in pureness, given fresh and milk total polyphenol content (b) from goats fed with the same forage species (modified from Ref. [48]). a, b, c, d, e, f = $P < 0.05$.

The results of a recent study [60] on three groups of *Girgentana* goats fed with Sulla fresh forage *ad libitum*, Sulla fresh forage *ad libitum* plus 800 g/d of barley meal and mixed hay *ad libitum* plus 800 g/d of barley meal indicate that Sulla fresh forage improves the plasma oxidative statuses of goats [61], milk total polyphenol (**Table 1**) content and the total antioxidant capacity of milk. Methods for milk total polyphenols and milk TAC assays are given in Section 2.2.3. Milk total polyphenol content seems closely related to its antioxidant activity. This fresh forage exerts antioxidant capacity due to its secondary compounds, which provide additional value in terms of oxidative status, and Sulla fresh forage seems to be a promising strategy for improving product quality.

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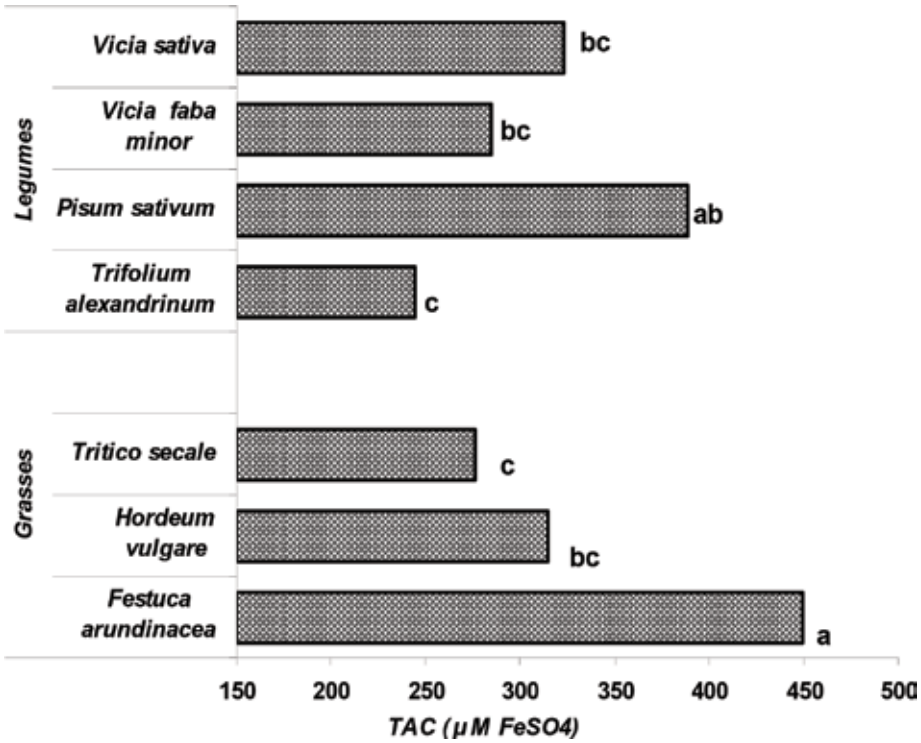

Figure 10. Comparison of total antioxidant capacity (TAC) in milk from goats fed *ad libitum* with a single species in pureness, given fresh (modified from Ref. [48]). a, b and c = $P < 0.05$.

	Feeding regimen		
	HB	**SUL**	**SULB**
Total polyphenol intake (g of GAE/d)	1.53[b]	9.20[b]	8.88[a]
Non-tannic polyphenol intake (g of GAE/d)	1.26[b]	3.72[a]	3.24[a]
Tannin intake (g of GAE/d)	0.26[b]	5.48[a]	5.64[a]
Condensed tannin intake (g of DE/d)	3.5[c]	47.2[a]	35.6[b]
Milk total polyphenols (g of GAE/d)	0.819[b]	0.964[a]	1.081[a]
Milk-free polyphenols (µg/mL of GAE)	49.3[b]	56.7[a]	56.2[a]
Total antioxidant capacity (log µmol/L)	2.38[f]	2.43[e]	2.47[e]

HB = mixed hay plus 800 g/d of barley meal; SUL = Sulla fresh forage; SULB = Sulla fresh forage plus 800 g/d of barley meal. GAE = gallic acid equivalent. DE = delphinidin equivalent,

[a–f] Values within a row without a common superscript letter are significantly different ($P \leq 0.05$).

Table 1. Daily intake of polyphenol compounds, milk content and total antioxidant capacity according to feeding regimen [60].

2.2.5. Case study 5: degree of antioxidant protection

In this case study, Ref. [13], in order to trace and identify milk and cheese from different feeding systems, proposed an interesting tool. Milk and cheese samples from ten feeding systems as grazing, grazing plus different types of supplement and indoor and zero grazing were studied to identify a tracing parameter correlated to the feeding system. In particular, α-tocopherol and cholesterol were measured in milk and cheese and were combined to calculate the degree of antioxidant protection (DAP). This tracing parameter was calculated as molar ratio between antioxidant compounds and a selected oxidation target. In dairy products from goats, only α-tocopherol was selected as the antioxidant because β-carotene is absent in goat's milk, and cholesterol was selected as oxidation target. All samples were analysed for α-tocopherol and cholesterol content. Briefly, all samples were hydrolysed in alkaline solution, and the extracted residue was dissolved in 2-propanol (1%) in n-hexane and analysed by the normal phase chromatographic method described in Ref. [13]. This index allows an evaluation of milk and cheese resistance to oxidative reactions, the main determinants of food quality and functionality for human nutrition. The DAP values (**Figure 11**) greater than 7.0×10^{-3} were found in grazing feeding systems, and values lower than 7.0×10^{-3} were found in indoor and zero grazing feeding systems, for milk and cheese.

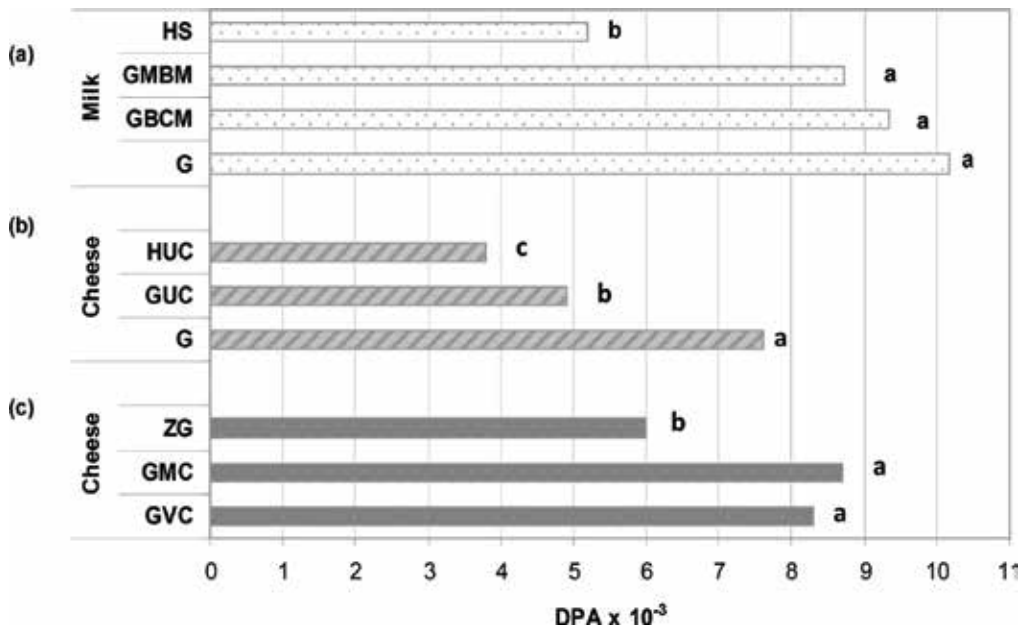

Figure 11. (a) Degree of antioxidant protection (DAP) of milk from goats fed with G = grazing, GBCM = grazing plus 0.6 kg/d mixed barley and chickpeas grain, GMBM = grazing plus 0.6 kg/d mixed corn and broad beans grain and HS = pasture hay *ad libitum* plus 0.6/kg/d of commercial concentrate. (b) DAP of *Caciotta* cheese from goats fed with G = grazing, GUC = grazing plus unlimited concentrate and HUC = hay plus unlimited concentrate. (c) DAP of *Caciotta* cheese from goats fed with GVC = grazing on valley pasture, GMC = grazing on mountain pasture and ZG = zero grazing (modified from Ref. [13]). a, b and c = $P < 0.05$.

These results show that cholesterol was highly protected against oxidative reactions when herbage was the only feed or was dominant in the goat diet. A strong positive correlation between herbage intake and DPA values allows to identify a linear regression: $y = 0.12x + 5.52$, where y = DPA ($\times 10^{-3}$) and x = contribution of grazed herbage intake to the animal diet calculated as a percentage of the maximum intake of mature Maltese goats (1100 g/d = 100% grazing). The DAP index equal to 7.0×10^{-3} was able to distinguish dairy products when the grazed herbage in the goats' diet exceeded 15%. The reliability of DAP to measure the antioxidant protection of cholesterol appeared more effective when the feeding system was based on grazing than when cut herbage or zero grazing was utilised indoors by animals.

3. The role of the breed on oligosaccharides: a special focus on the Mediterranean goats

Besides the feeding system, the breed plays a fundamental role in affecting the nutritional profile of goat milk and cheese. The breed may be considered the result of the adaptation of a species to a given environment, basically in order to go over the climate and feeding and water resource limits that might affect the reproduction and kidding. The goats are present in high mountains as far as in the internal lands and coastal regions; they are reared in technological farms but also in extensive, grazing systems in the Mediterranean area, an environment characterised by high variability, that was able to select very different breeds [62].

The so-called native breed has become able to optimise the resources in terms of water and feedstuff [63]. The breed's answer is expressed as phenotype, quantity and, overall, quality of production. The differences are both in micro and macronutrients, and they are affected by the environment directly or indirectly. In the first case, we can say that different breed means different feeding behaviour and thus milk yield and quality, since it is well known that feeding largely affects the milk composition [64]. Moreover, the genetic polymorphism may affect the milk features.

Within the same breed, in the same environment and diet, it is expectable to have very similar performances. Contrarily, especially for goat, significant differences have been found for quality but also quantity parameters. This variability has been explained, in part, by the genetic polymorphism of caseins that are α_{s2}-casein, β-casein and k-casein but in particular at the locus α_{s1}-casein, first discovered by Boulanger et al. [65]. It was found that goats carrying strong alleles (AA) for high α-s_1 casein present higher percentage of milk casein, fat, calcium, phosphorus and smaller micelles than the milk from goats with weak alleles (FF) [66, 67]. Several goat breeds have been characterised for this variability: the Vallesana, Roccaverano, Jonica, Garganica and Maltese breeds [68] and Alpine breed [69]. Spanish Malagueña goats with a high (HG) and low (LG) genetic capability for α_{S1}-casein synthesis were used to determine whether the different genotypes were related to differences in feed utilisation (13.6 vs. 17.7% crude protein content for diets 1 and 2, respectively). The findings have let to explain the differences in milk composition between the two genotype groups by the greater nitrogen and energy utilisation of HG vs. LG goats [70]. Moreover, the interaction genotype x feeding system was studied (e.g., see Ref. [71] on Malagueña dairy goat breed).

The breed effect on milk oligosaccharide (OS) composition, and in particular sialyloligosaccharide (SOS) content, is scarcely studied. The milk from the Spanish Murciana-Granadina goat breed was found characterised by 25 OS [72], later [73] isolated 15 new oligosaccharide structures from fresh milk of Spanish goats, obtaining a virtually lactose and salt-free product, containing more than 80% of the original oligosaccharide content. Evenly, the effect of interaction of breed x feeding is scarcely studied.

Within the management's strategies, the choice of the breed is a key element to weigh up towards the type of livestock, the available resources and the business plan, in terms of destination of the milk, namely, if destined to the market of drinking milk or to dairy production. Some speculations can be made whether optimizing production, rheological properties, and bioactive profile and content may be feasible with feeding management modulated in terms of energy and protein supply depending upon genotype. The following three case studies are presented, in order to partially cover this gap on Mediterranean goats.

3.1. Case studies 1 and 2: oligosaccharides in colostrum and milk

A study on the content of three SOSs, namely, 3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL) and di-sialyllactose (DSL) in colostrum and milk, was carried out [22] with two Italian goat breeds, Garganica goat (a native breed from Gargano Mountain in Apulia region, Southern Italy) and the Maltese goat breed, native from Malta isle in the Mediterranean area. The animals were fed indoor, receiving hay (from polyphytic cultivated meadows) *ad libitum* and concentrate supplementation (400 and 600 g/h/d, respectively) at 14% crude protein, according to their milk yield (800 and 1200 g/day milk, respectively). The SOSs were isolated from individual colostrum and milk samples obtained in five periods (at kidding, 24 h, 7 days, 30 days and 90 days after delivery). Briefly, after centrifugation (2000 × g, 4°C, 10 min) the supernatant lipid layer was removed, and the proteins were precipitated by addition of 0.5 volumes of 1.8 g 100 mL^{-1} $Ba(OH)_2 \cdot 8H_2O$ and 0.5 volumes of 2 g 100 mL^{-1} $ZnSO_4 \cdot 7H_2O$. The blend was vortexed and centrifuged (12,000 × g, 10 min, 4°C). The supernatant was removed and centrifuged again. The second supernatant was filtered with a 0.45 μm nylon filter prior to analysis by high-performance anion-exchange chromatography (HPAEC) on a Dionex PA100 column (Dionex, Sunnyvale, California, USA). Elution was monitored by pulsed amperometric detection (Dionex ED40) and the gradient controlled by a Varian ProStar pump system. Data were collected and analysed by Star Chromatography Workstation 6.41 (Varian, Inc. Walnut Creek, California, USA), and 6′-SL, 3′-SL and DSL external standards were used to generate standard curves for comparison.

The results showed a significant effect of breed and sampling time on SOS content. Garganica breed showed the highest values of 3′-SL and 6′-SL while Maltese breed the lowest content of DSL (**Figure 12**).

Also the interaction breed x sampling time affected the SOS content in milk and colostrum; in particular, 3′-SL content was significantly higher in Garganica colostrum at 24 h after kidding and in milk at the 7th and 30th day. DSL was affected by interaction, showing higher values in Garganica's colostrum at parturition and Maltese's colostrum 24 h and milk. The content of the

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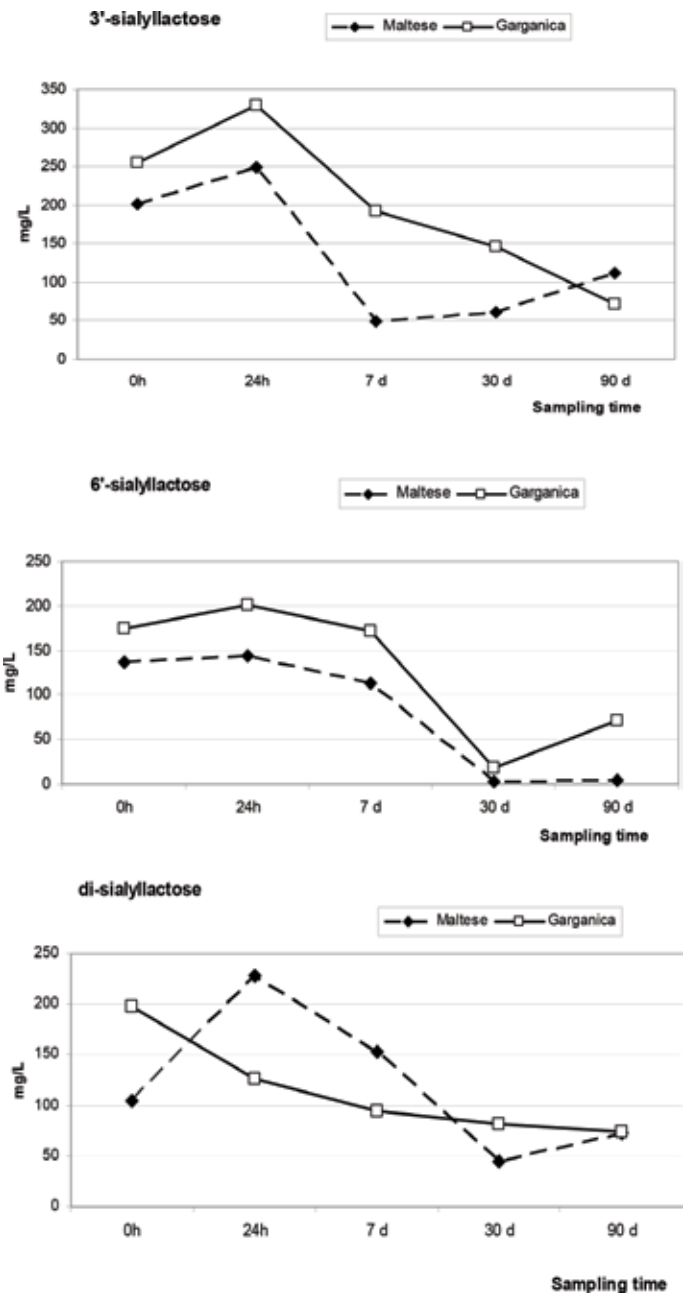

Figure 12. Effect of breed and sampling time on SOS concentration in milk [22].

three SOSs was higher than values found by Ref. [73] on Spanish goats, confirming the breed effect and, consequently, the importance of the choice of the breed among the management options aimed at improving the nutraceutical quality of milk, namely, the oligosaccharide content.

In a further study [74], Garganica goat milk SOSs were compared with Saanen goats' from colostrum time to the 90th day after parturition, in their 3rd parity. In the experiment, the Saanen and Garganica goats were fed indoor, receiving hay *ad libitum* (50% polyphytic meadow and 50% alfalfa hay) and 700 g/h/day DM and 450 g/day/h DM, respectively, of concentrate supplementation at 18.3% crude protein (wheat by-products, corn grain, soybean meal, molasses, supplemented with mineral mixtures). The results confirmed a breed effect on SOS contents in colostrum and milk also between Garganica and Saanen breed, where Garganica goats showed mean higher values for the three SOSs (see **Table 2**).

Moreover, a significant interaction breed x sampling time was recorded for 3′-SL ($P < 0.001$) and 6′-SL ($P < 0.01$), while no significant interaction was found for DSL. The results may be considered under a genetic point of view. In fact, under equal feeding condition, the breeds expressed their OS synthesis potential, probably influenced by the genetic polymorphism in the locus CSN1S1 (α_{s1}-casein). On this matter, the Saanen goats were characterised by a high frequency of defective alleles (F and E) and low frequency of strong alleles (A and B) at α_{s1}-casein locus; contrarily Garganica goats had high frequency at strong alleles and low frequency at weak alleles (F) [75].

In a previous study, Ref. [76] have found in Alpine goats that the genotype (A/A or 0/0) affected the OS profile, even though not the total OS production. So, Claps et al. [74] speculated that an indirect link between goat breeds, about the allelic frequencies at the locus α_{s1}-casein in Saanen and Garganica goat breeds, might have affected the SOS content in milk and colostrum, where the Saanen breed, characterised by high frequencies of defective alleles [66], could have adversely affected the production of SOSs.

3.2. Case study 3: interaction of genotype with feeding regimen

The Mediterranean Red goat was characterised for the content of three SOSs considering the polymorphism at locus CSN1S1 and its interaction with feeding regimen [77]. Six goats, with genotype A/A (strong, α_{s1}-casein producers), and six goats with genotype F/F (weak) were fed with two diets in pellet, respectively, at 100% of energetic and 105% of protein requirements (M) and 70% of energetic and 75% of protein requirements (L). Milk samples at the 69th ± 3 day of milking were analysed for the content of three sialyllactoses (see Section 3.1),

SOS	Garganica	Saanen	SE	Significance
3′-SL (mg/L)	195.5 a	124.8 b	12.7	***
6′-SL (mg/L)	129.7 a	15.4 b	5.5	***
DSL (mg/L)	104.0 a	79.9 b	7.3	*

Means within a row with different letters (a, b) differ at $P \leq 0.05$. SE = standard error.

*$P < 0.05$.

***$P < 0.001$.

Table 2. Mean content in milk of 3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL) and disialyllactose (DSL) from Garganica goat breed compared with Saanen, a cosmopolitan breed, at 30 days in milk (from Ref. [74]).

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considering the genotype, the diet and their interaction. The results revealed that genotype and diet affected the 3′-SL content in milk ($P < 0.05$) (**Figure 13**), while their interaction expressed only a trend of variation ($P = 0.10$). The goats fed with undernourishing diet (D) showed a 3′-SL content 58.5% lower than M goats.

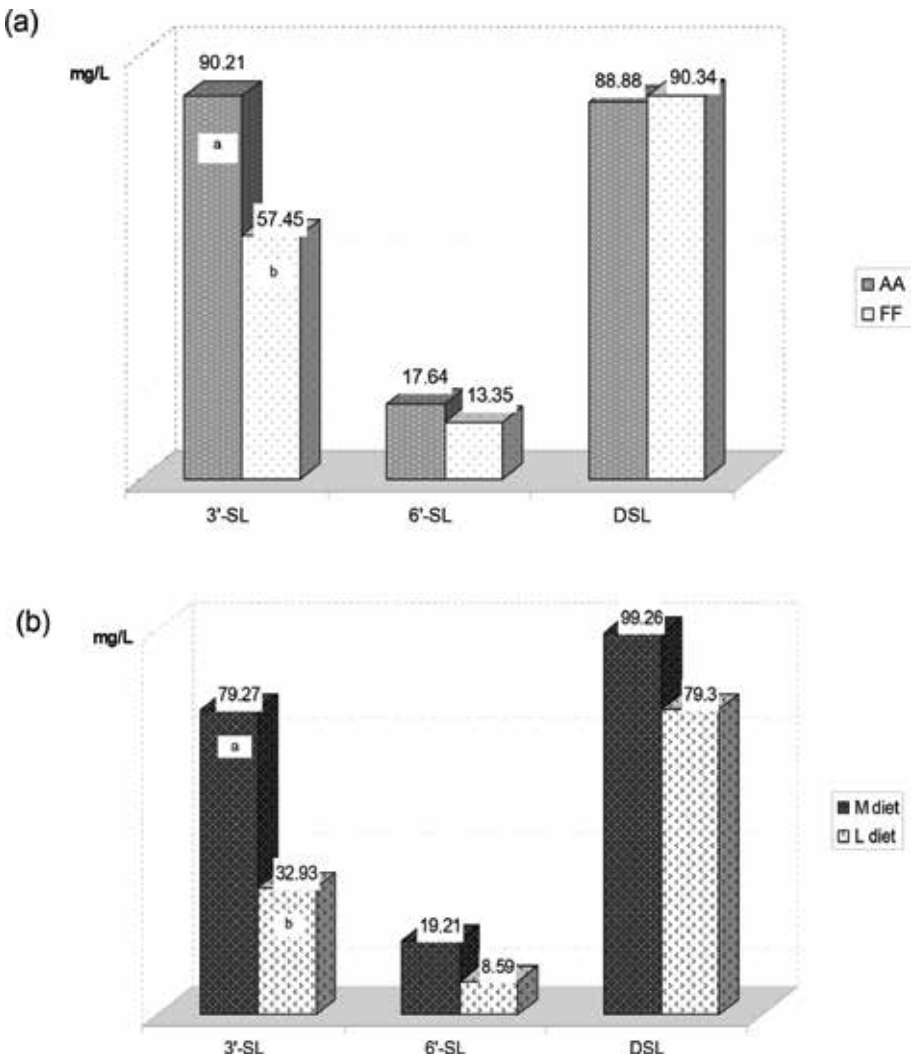

Figure 13. Effect of genotype (a) and diet (b) on three sialyloligosaccharides in Mediterranean Red goat milk (adapted from Ref. [77]).

The 6′-SL and DSL showed only a decreasing trend. This result might be related to the reduction of the expression of genes involved in the milk synthesis after a prolonged fasting [78]. Similarly, in human milk a decrease of OS was found in milk from undernourished women [11].

These results demonstrated that there is a different efficiency in diet utilisation and response in synthesis of metabolites such as oligosaccharides, depending on the genotype. Consequently, in systems that use selected animals, the diet may be formulated taking into account the genotype, in order to achieve certain qualitative profile of goat milk and increase the efficiency in feeding management.

4. Conclusion

The case studies discussed along with the literature review contribute to widen the farmers' basis for choosing the breed and the feeding regimen by including management decisions specifically aimed at improving milk and cheese nutraceutical properties. The management options considered hereby are mainly conceived for Mediterranean extensive and semi-intensive goat production systems but can easily lend themselves to other ruminant and non-ruminant production systems. This analysis can help farmers to improve milk nutraceutical composition in a sustainable and low-cost way by partially replacing expensive commercial concentrate with farm-grown forages and natural pasture grazing. Data on the nutraceutical profile of milk produced by Mediterranean breeds, as well as on the use of farm-grown forages, open new alleys towards the potential role of local breeds/forages for developing innovative and sustainable health-promoting dairy supply chains.

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Goat's Meat

Chapter 12

Carcass and Meat Quality in Goat

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Additional information is available at the end of the chapter

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Abstract

Goat is a worldwide spread species with different specialities and aptitudes, among the meat production. Its consumption varies widely depending on the region of the world considered. However, a common factor is the presence of few studies in comparison with ovine, especially those that characterize the quality of its products (related to carcass and meat). Generation, availability, and diffusion of characteristics of the species and its production, generated from scientific studies, could help breeders and society on their education and raising global awareness of its importance, conservation, and productive possibilities. Goat has its own specific characteristics related to quality with a presumed good acceptability of its products by consumers. On the current chapter, the effects of the main factors that modify carcass and meat quality in goat are compiled. Both, intrinsic factors (breed or breed type, age, weight at slaughter or gender) and some extrinsic factors (as production system, type of suckling, and aging) are discussed.

Keywords: acceptability, instrumental analyses, kid, proximate composition, sensory

1. Introduction

Goat is the most widespread livestock species, as [1] reported and listed. There is an important visible genetic variation on goat species, with approximately 570 different breeds, with their own morphological characteristics, productive performances, and adaptation to specific climate and environment. Breeds are classified in 5 specialities: meat, milk, prolificacy, fiber (pashmina—cashmere and mohair), and skin. Currently, there are more than 1 billion heads around the world [2], with 57.4% of the census located in Asia, 37.0% in Africa, and 3.5% in America, representing Europe 1.6% and Oceania 0.4%. Over the last 50 years, as [3] compiled, goat population has multiplied by 2.4 times while other livestock species have maintained it or reduced. Nevertheless, goats in most countries are kept in small herds, not always very

profitable and in various aspects dependent on different monetary or political supports from governments or local entities, as it was concluded in the 2014 European Regional Conference [4]. Goat has demonstrated to be a really important livestock in different parts of the world, mainly due to its versatility and high adaptation's capacity to different production systems from the high intensification, in the most developed countries on dairy herds, to the hard conditions of arid areas. On the other hand, some studies have shown that a correct management of goats on grasslands can also help to increase plant biomass and biodiversity [3], having also a social function by fixing people to the territory, avoiding more movements to big nucleus-cities, and consequently depopulating the rural areas.

To support goat production is necessary the generation, availability and diffusion to the people, of new and diverse information related to characteristics of the species, possibilities of production, product quality and its benefits for the humanity's future [4]. As already [1] pointed in the last decade, the possible benefits from science advances on several fields, considering goat, are not yet so extent as on other livestock species, although that scientific generated information could help breeders and society on their education and raising global awareness of the species. Because the knowledge generated can be applied on everyday management, building a more sustainable and prosperous future on goat. With this aim of spread knowledge and application of the science, in this chapter, factors that affect carcass and meat quality will be analyzed, mainly focusing on the expertise and experience obtained from experimental studies and results developed by the authors.

2. Material and methods

In general a standardization of methodologies in any field of research would be necessary. This would allow the creation of universal and homogenous databases, letting the comparison of studies and their diverse variables in a global way around the world. This idea is more than a necessity in the twenty-first century.

Related to carcass quality in small ruminants, it is fundamental to compile some basic information about animals, as age and weight, that let an official classification according to the different categories existent depending of each State legislations. Afterward, an external classification according to morphology and fatness is necessary, using the official European Systems based on photographic models [5, 6]. Also morphological measurements, as those suggested by [7–9], help to compare carcass characteristics, in an objective way, including compactness indexes of the studied animals. In the same way, a standard protocol for cutting and dissect representative parts of the carcass (shoulder or leg) could be important, as those reported by [8, 10], to obtain information about tissular composition and the real value of commercial cuts.

Evaluation of meat quality can be defined by different attributes or variables. Firstly, monitoring pH with a commercial pH meters is necessary to verify the absence of alterations in this parameter, a fact that would modify other meat quality variables. An objective definition of color using the CIEL*a*b* system with diverse types of spectrocolorimetres let define color in some basis variables as luminosity (L*), yellowness (b*), redness (a*), tone (H°) or chrome (C*). The standardization of measuring conditions, as instance blooming, should be indicated.

Texture analyses are assessed by texturometers which have different blades that let obtain mainly values related to shear force or resistance to compression of meat samples (raw or cooked). Some standard protocols to determine texture have been proposed by Honikel and Lepetit and Culioli [11, 12] and summarized in [13]. Proximate composition of meat can be defined by the quantification of its different components such as moisture, ash, protein, or fat evaluated according to International Organization for Standardization Protocols (ISO). For the fatty acid analysis, intramuscular fat can be extracted by various protocols as those suggested by [14] and after the methyl esterification samples are analyzed by gas chromatography. Related to sensorial analyses, a trained panel let obtain objective scores of the sensorial attributes of meat [15] and the use of different kinds of consumer hedonic test show the acceptability of consumers related to the product [16]. Detailed information about different methodologies cited in this section and along the chapter can be consulted in [17–19].

3. State of the art: goat meat production and some general concepts about product quality

The billon of goat census by FAO on 2014 [2], produced 5,524,075 annual tonnes of meat. According to the world distribution of goat, the main meat production occurs in Asia (71.9%), followed by Africa (23.5%), America (2.2%), and Europe (1.9%), being the production in Oceania only 0.5% of the total meat goat production. Classifying those data by countries, the top five producers currently are: China (2,098,100 t), India (505,064 t), Pakistan (309,000 t), Nigeria (244,575 t), and Bangladesh (208,613 t). In Europe, the situation has not changed too much in the last years [3], with a low goat meat production compared to the previously described top five, being Greece the country with the largest meat production (23,893 t), followed by Eastern European countries such as Russian Federation (17,515 t), Albania (14,850 t), and Romania (9126 t); France and Spain also presented a significant production with 12,077 t and 8010 t, respectively.

The differences between the commented countries or world regions, can reside in the own use and habits of consumption of goat meat. Especially for consumers in developing countries [20], goat food products represent an important nutrient source, being this species an important part of the habitual diet of the population, as well as an important resource for income (until more than 70% of the total on small holder farms [1]). However, for Western consumers, goat food products (particularly dairy products) are considered luxurious, and goat meat consumption has a low frequency rate, being mainly associated to punctual-festive events, specific regions, or associated to label quality brands as a high-quality difference product.

The concept of quality is complex and dependent on the aspect that it is considered. Usually, quality is defined as "All those attributes for what consumers are willing to pay more," or an extra in the base prize in order to have some specific attributes guaranteed. Quality can be associated to different aspects as nutritional attributes (low fat content or healthy fat profile), production system (sustainable, organic or welfare friendly, for instance) or particular sensorial attributes (optimal odor, texture or flavor and, at the end, some extra hedonic satisfaction). Many of these aspects could be related with certain quality labels that support or guarantee the extra paid quality.

To determine quality in relation to meat, we should focus on a specific market [21]. Meat quality needs studies in depth, being a complex topic which presents a lot of questions and aspects to be dealt both, in general (animalism, sustainability, and human health) and with the goat meat in particular (habits, prices, problems of availability and a lack of culture about its specificities and own characteristics). Some of these points (all related with the culture, information, and adaptation to some tendencies of the new markets) could be satisfied with information and adequate labels. The label is the industry and producers' way of communicating directly with the consumer. It should be attractive with a good design, and if possible interactive, providing information on the product's origin and background, its nutritive and sensory qualities and veracity between the written and the reality [21].

At the end, increasing demand and improving marketing for meat should entail increased production, which must be worthwhile for producers, sustainable for the planet, economically profitable for the production chain, of healthy quality, as well as affordable for consumers [21].

4. Main factors that affect carcass and meat quality in goat

There are several factors that affect the final quality of the product related to carcass and meat. Those, as it is compiled in **Figure 1**, can be classified as intrinsic or extrinsic [22].

Due to the high quantity and variability of factors, only some of them will be discussed in the current chapter, those which usually produce more variations. Thus, as intrinsic factors,

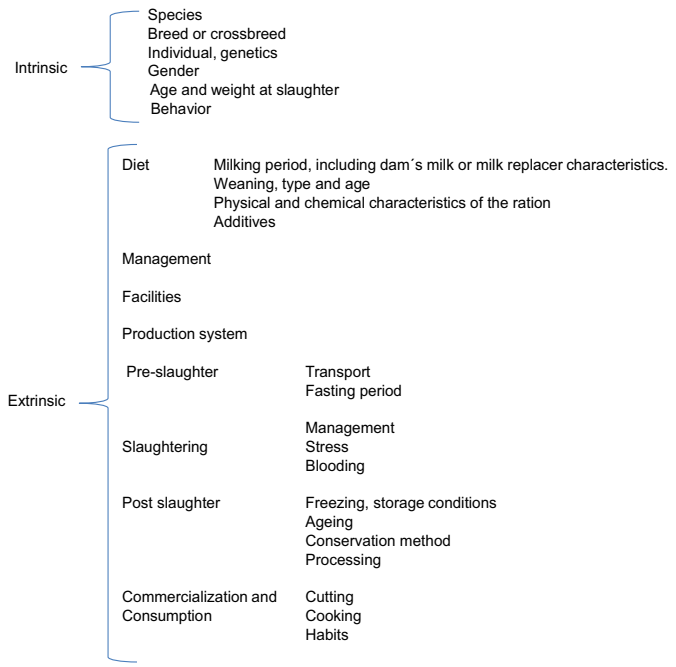

Figure 1. Main factors that modified carcass and meat quality in goat. *Source*: Adapted from Ref. [22].

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we will analyze **breed** or breed type (dairy vs. meat aptitude, rustics vs. specialized breeds), **age or weight** at slaughter, and **gender** and, as extrinsic factors, we will analyze the effects of **production system**, type of suckling (dam's milk vs. milk replacer), and post-slaughter **aging**.

5. Carcass quality

Carcass quality parameters are mainly divided on those that affect morphology, as conformation and morphological measurements, and those related to composition as commercial cuts, fatness score or tissular or chemical composition. Tissular composition is usually obtained as percentage of tissue components from the complete carcass or from specific commercial cuts after a standard cutting and dissection [10, 23].

Carcass traits, such as the conformation as well as fat distribution within the carcass, have a great importance in meat production, because of their economical implications. The proportion of high value cuts is an indication of its overall value, having, some carcasses attributes concerned with the quantity of saleable meat, significant implications on the technological properties of the meat, i.e., the morphology of some specific muscles and cuts [24].

5.1. Weight, conformation and morphological measurements

Average carcass weight differs depending on the continent or country considered, being 12 kg a world average, and 10 kg for Europe or Spain [1]. Carcass performance on suckling kids of 6–10 kg of life weight can present values between 60 and 65%, because in young animals, only skin and white offals are excluded. In old animals, head and red offals (liver, hearth, spleen, lungs, etc.) are also excluded, presenting a carcass performance of around 50%.

Conformation is used to describe carcass morphology and the visual impression that the different categories produce on the observer [25]. It can be assessed in a comparative way with photographic models, or using measurements based on different anatomical points. For goat species, it does not exist an official classification system of carcass quality, as happens in other ruminants (European System SEUROP for conformation), although some authors have done some proposals [23, 25, 26]. In Spain goat, carcass is classified by animal age as: suckling kid (younger than 1.5 months), young goat (between 1.5 and 6 months of age), and adults (older than 6 months).

Some studies have showed that carcass quality can differ significantly among **breeds**, but differences mainly depend on the criteria used in the comparisons (same weight, same age, or same proportion of mature weight). The results will be different depending on the comparative criteria employed.

For example, at commercial **slaughter weights**, unweaned kids from dairy or meat breeds reared on their dam's milk, following the local husbandry practices that differed significantly in conformation notes and morphology variables [24]. Average carcass weights ranged from 4.38 kg (dairy breed) to 6.56 kg in some meat breeds. Also, products from dairy breeds presented lower conformation (1.70 in a 15 points scale: from 1 poor: poorly muscled carcasses of inferior shape, to 15: excellent, carcasses of outstanding shape) than those from meat breeds.

Related to kid morphological measurements, when data from Ref. [24] were covariated by the carcass weight, smaller differences were appreciated between dairy and meat purpose animals. Thus, some meat breeds presented the longest pelvic limb, but dairy or meat breeds did not statistically differ either on carcass external length, buttock width or chest depth, showing small differences in morphology, independently of their main aptitude. This applies to the most local breeds, which are not meat specialized as Boer, at young slaughter ages.

According to data compiled by [27], which show more than 60 different **commercial types** from several locations around the world, age, and live weights, differences between breeds on carcass yield are associated to the final size of the breed, degree of fatness, and aptitude. The lowest performances have been reported in animals of 120–180 days of age, from Chilean Creole breed, with a 45.1% carcass yield [28], and the highest at young Gigertana kids of 25 days of aging with a 70.6% carcass yield [29]. On the other hand, from a commercial point of view, an increment on slaughter weight from 4 to 6 kg may not improve cold carcass yield in Serrana meat breed [27].

Age and, consequently, maturity are the most important factors that modify carcass characteristics [30]. Final carcass weight increased on the meat purpose breed Serrana in kids from 10 to 40% of the final adult weight (FAW); however, the performance of 20 and 30% were lower (46.2–46.6% respectively) than those obtained at 10% (51.4%) or 40% (47.9%) of the FAW. On the same study [30], the **gender** had a significant effect on live and carcass cold weights; however, no differences were reported on carcass yield. In Ref. [31], it was found that males presented the greatest weights and average daily gains related to females, especially when came from 3 to 5 years old animals, as well as in single born animals and in autumn.

Different breeds produce differences in morphological measurements ranging thoracic depth from 15.7 cm to 31.4 cm or buttock perimeter from 42.6 to 53.0 cm, being not homogenous the effect of the gender on those variables [27]. Also, results [27] showed that an increment in slaughter weight also presents an increment in morphological measurements of the carcass, which improve carcass compactness index (carcass weight/length carcass), although without effect on leg compactness.

5.2. Carcass composition and fatness

For fatness score, a classification has been developed with a 5 points scale from 1 (very lean or very low fat cover) to 5 (when the carcass is completely covered) [25].

Breed has been described as an important effect in composition and fatness. Related to the fatness score described in [25], a Spanish meat breed such as Blanca Celtibérica presents the highest fatness score [24], because it is normally consumed at heavy **slaughter weights** (6.56 kg cold carcass weight-CCW). However, within suckling animals (the most consumed in Spain), some dairy breeds (Murciano-Granadina) show higher scores (highest fatness) than local meat purpose breeds, even when those parameters are covariated by CCW, showing their highest precociousness.

According to Ref. [10], carcass in small ruminants can be divided into five main regions corresponding to different commercial cuts: leg, ribs, flank, shoulder, and neck. Some results obtained from several studies with cold carcass weights from 3.2 to 16.0 kg are compiled in **Table 1**, being the variability mainly dependent on the studied breed and slaughter weight.

According to allometric coefficients [32], when the weight of an animal increases, there is a decrement in the percentage of bone and an increment in the percentage of fat, being the muscle kept almost constant. Early developed cuts are the leg and shoulder, being ribs and flank the late developed cuts [27].

Tissue composition highly affects commercial quality of the carcass. As happens in other species, as ovine, consumers pay the same price for fat, muscle, and bone, all inclusive in the commercial cuts. Due to this, fat composition is an important factor to consider. It has been reported a great variation on the composition between goat **breeds** [10, 24]: muscle between 69 and 50%, 34–11.8% for bone (a high rate correspondent to young animals), and a percentage of fat between 23.7 and 5%, that could be considered, in general, as low, but it depends of the age, breed and, obviously, fatness score. A dairy breed such as Murciano-Granadina presents the lowest subcutaneous fat percentage, although not different from the local meat purpose breeds, reflecting the tendency for dairy breeds to store more visceral than carcass fat. This visceral deposit has been described as precocious, and it might develop quicker because of the high nutritional level of dairy kids that results from their high dam's dairy production potential [24].

In Ref. [33], it has been described that natural **suckling** gives higher fatness score and weight of renal fat, compared to kids fed with milk replacer. These results suggest a better assimilation of the natural than artificial milk, although it could also be associated with differences in composition between milks.

On meat aptitude goats, Ref. [27] found a stronger effect of **age** or **final body weight** than **gender**. With the increase of body weight (carcass weight from 4 to 8 kg), fat percentage increased 4% and bone percentage decreased the same amount, having females almost 2% points more of fat and less of bone. No differences in muscle percentage were reported. Consequently, relation muscle/bone was better for female than male kids.

Cut	Variability (%)
Leg	33.6–26.4
Rib	32.5–21.7
Flank	13.6–9.5
Shoulder	25.7–17.9
Neck	12.6–7.4

Source: Adapted from Ref. [27].

Table 1. Percentage of different commercial cuts on goat carcasses.

In general, it could be said that goat carcasses are longilinear and, consequently, they have a poor conformation. Also, they have high muscle and bone percentages and low content of fat, especially subcutaneous fat.

6. Meat quality

6.1. pH

pH can be considered one of the most important and basic factors that can affect meat quality. Nevertheless, goat is a species in which alterations with low pH (PSE) or high pH (DFD) are not usual.

Several studies have reported differences in pH between **breeds** but probably more associated to differences in the management previous to the slaughter than to the own breed. Thus, Colomer-Rocher et al. and Ripoll et al. [24, 34] at same **weight** and **management** reported statistical differences between breeds. However, these differences can be considered insignificant (5.76–5.80). In Ref. [13], slight differences that are dependent on carcass weight, where pH was lower on heavier carcasses (8 kg—5.8 pH) respect to less mature animals (4 kg—5.9 pH), were reported. Gender usually does not modifies pH [27, 35].

6.2. Color

Color is an important attribute that affects consumer purchase and willingness to buy. Preferences for a specific color (paler or darker) depend on the type of consumer considered (usually conditioned by the nationality, cultural background and experience or consumptions habits [36]).

Some differences have been reported among **breeds** [37], due to the different aptitudes of the breeds or crossbreds compared: fiber, dairy, and meat. The highest luminosity (L*) have been reported on crossbred meat x dairy genotype (Boer × Saanen) and the lowest redness (a*) and yellowness (b*) for meat and wild goat crossbred genotype (Boer × Feral).

In Ref. [34], differences in L* between breeds on light weight animals were not reported. Obtained values and differences can be considered low and explained by the milk-based diet of young animals, since milk is not rich in iron which would darken the meat. However, [38] found differences between kids from dairy or meat aptitude, presenting dairy animals breed (Murciano Granadina) significant higher L* and H° (almost 10 points) than those from meat aptitude (Bermeya), without differences on b* between breeds.

In **Table 2,** some color variables of different studies are compiled, showing the variability on this parameter depending on the factors considered.

Related to **age** and **weight**, the results from Ref. [27] showed that color of *Longissimus* was statistically modified. Increasing CCW (4–8 kg) produces a progressive reduction of luminosity (L*: 49.0–43.6), and an increment of redness (a*: 9.4–13.8) without variation of

L*	a*	b*	H°	C*	Breed	Weight (kg)	Reference
55.1–49.1	13.4–10.6	5.8–5.3	28.7–23.4	14.6–12.1	Spanish kids (meat and dairy apt)	4.3–6.5 CCW	[24]
55.2–52.9	11.7–9.3	20.9–14.5	62.1–54.4	23.6–18.0	5 Spanish kids meat apt.	7.4–8.0 SW	[34]
53.4–48.3	13.8–9.2	19.3–12.9	59.9–44.3	22.0–17.7	5 Spanish kids meat apt.	10.9–12.3 SW	[34]
49.0–43.6	13.8–9.4	10.0–9.3	46.4–35.1	16.8–13.6	Portuguese kid meat apt.	4–8 CCW	[27]
43.6–37.7	12.4–10.3	8.1–6.7	35.4–31.2	14.5–12.4	6 crossbreeds different apt.	11.8–10.9 CCW	[37]

CCW, cold carcass weight; SW, slaughter live weight.

Table 2. Color attributes of goat's meat from different studies.

yellowness (b*) with values between 9.3 and 10.0. Differences in color are probably associated with the increment of myoglobin because the concentration of this pigment has been demonstrated that increases with the animal age, increasing color intensity [27, 39].

Similar results were reported in [34], where heavy weight kids (11 kg live weight) presented almost 3–4 points lower luminosity than light weight kids (7.6 kg LW); however, redness was not modified and b* decreased only in some meat breeds, without modification in other meat purpose breeds. Guerrero et al. [38] on meat breed Bermeya slaughtered at 7 kg or 10.5 kg CCW, only reported differences in redness and Chrome variables which increase with the **slaughter weight**. Then, in general terms, L*, b*, and H° diminished, and C* and a* increased their values with the increasing slaughter weight.

As happened in pH, no differences in color have been associated to **gender** [27, 40].

Related to productive **system and age**, Dhanda et al. [37] reported differences between meat purpose goats, from suckling kids and low slaughter weights (Capretto 6–12 kg) to older goats with carcass weight of 16–22 kg. Total pigment concentration in the *Longissimus* muscle was significantly higher in Chevon compared to Capretto carcasses, and a modification in color intensity is expected. However, only differences in b* were reported in the cited study, explained as a difference of slaughter age (5 months).

6.3. Texture

Aging is the most important factor that modifies meat texture and consequently eating quality, consumer acceptability, and satisfaction. The metabolic-biochemical reactions that happen after *rigor mortis* let a progressive tenderisation of the meat [41]. Tenderness can be evaluated instrumentally by texturometers or by sensory methodologies. In this section, only instrumental characteristics will be assessed. As happened in lamb, the period comprised from 3 to 8 days of aging seems to be enough to reach a desirable tenderness without damaging sensory perception [16, 22]. It is important to consider this factor (aging) and try to isolate it (using the same meat aging conditions on the comparisons) to understand the effect of other factors on texture variables.

Usually, as happens in other ruminant species, **breed** modifies texture parameters, especially when different aptitudes are compared. This happened in Ref. [37], where after 24 h of aging, a crossbred with a meat breed (Boer × Feral) presented significant lower shear force values (3.7 kg/cm^2) than a dairy crossbred (Saanen × Feral, 4.6 kg/cm^2).

In Ref. [34], it was reported that the three meat compression variables studied (C20%—related to myofibril component, C80%—related to connective component-collagen and the maximum compression ratio C100%—the force necessary for the total compression [12]), were affected by both **breed** and **slaughter weight** factors. Differences of almost 5 N/cm^2 were reported between different meat breeds at 20% of compression, 16 N/cm^2 at 80%, and 23 N/cm^2 at 100%, being the maximum values of 14.96, 79.74, and 101.70 N/cm^2 for C20, C80, and C100%, respectively.

In the cited study [34], aging of samples was 3 days. The higher myofibrillar toughness of light kids could be explained by a lower activity of muscle proteolytic systems, as well as a lower rate of post mortem tenderisation, or higher myofibrillar density due to the short age of light kids. As a general rule, by increasing the aging time, tenderness will increase [42]. Tenderizing is more intense in older animals due to the higher action of the proteases.

Results from Ref. [38] reported that breed was only a significant factor of variation at short aging periods (2 days) on C20% variable, where meat from a dairy breed was tougher than those from meat breed kids at both slaughter weights (light or heavy carcasses). However, at 4 days of aging, those differences between breeds disappeared, being most significant the effect of aging tenderizing than the effect of the breed purpose.

According to Ref. [40], **gender** did not affect texture parameters also after 3 days of aging, in which medium values are between 8.3 and 8.4 kg/cm^2 on shear force.

6.4. Proximate composition and fatty acid profile

Goat meat can be considered as red meat, as those from other small ruminant species, according to its *proximate composition*. As some authors [20] have pointed before, meat from goat has a high nutritional value, contributing to an enjoyable and healthy human diet.

Goat meat compared to the other ruminant meats is characterized by a higher water content, lower energy contribution as well as lower fat content, with a similar proportion of minerals (**Table 3**). Values are dependent on the source consulted (USDA; BEDCA). The type of animal and the piece used in the determination of proximate composition explain the variations reflected in **Table 3**.

In Ref. [34] were not found differences between **breeds** on chemical composition neither on light or heavy kids, being comprised between 78.01 and 76.97% for moisture, 24.11 and 19.52% for crude protein, 1.11 and 1.03% for ash and 2.05 and1.09% for fat. However, the results from Ref. [38] showed that differences between aptitudes exist specially in fat percentage, where commercial dairy kids presented higher percentage (7.49% vs. 1.9–2.8%) than kids from meat aptitude even at lower carcass weight.

	Goat [43]	Lamb [43]	Kid [44]	Lamb [44]
Water, g	75.84	71.40	64.50	64.10
Energy, kcal	109	142	225	242
Protein, g	20.60	19.98	17.11	15.60
Total lipids (fat), g	2.31	6.88	17.49	20.10
Minerals				
Calcium, mg	13.00	18.00	9.56	9.00
Iron, mg	2.83	1.52	0.57	2.60
Magnesium, mg	—	23.00	15.82	16.00
Phosphorus, mg	180.00	189.00	156.77	170.00
Potassium, mg	385.00	327.00	248.93	320.00
Sodium, mg	82.00	77.00	64.06	75.00
Zinc, mg	4.00	2.69	2.24	2.20

Source: [43]: goat raw (Ref. 17168); lamb, New Zealand, imported, loin chop, separable lean only, raw (Ref. 17078). [44]: milk kid ribs—Murciano-Granadina (raw); lamb ribs (raw).

Table 3. Basic composition characteristics of different ruminant species. Values per 100 g of an edible portion.

Slaughter weight also did not affect proximate composition [34], only fat percentage for the meat breed Pirenaica, which proportion decrease with the age of the animal from 2.05 to 1.37%. Those results from Ref. [34] agree with others [29, 45] were slaughter weight did not affect the chemical composition of the loin in young animals. Usually ash and fat content is low on kid meat, and main differences between breeds on chemical composition are associated to intramuscular fat. However, [38] reported small differences between slaughter weights on other chemical components, where animals from heavy carcass presented lower moisture percentage (75.7% vs. 77.8%) and higher protein content (18.8% vs. 17.1%) than light kids (which had 3.5 kg less of carcass weight).

Gender was unaffected by dry matter and fat percentage in kids with CCW of 4.5–5.0 kg; dry matter was reported as 24.1–23.8% in females and males, respectively, and the fat content 1.7–1.4% [40], similar to other breeds (meat aptitude).

Fatty acid composition is an important attribute to be considered by their implication in human health. Also fat composition affects organic attributes, especially those related to flavor, and consequences of lipid oxidation (rancidity).

In Ref. [46], the fatty acid profile of three different depots (intramuscular, subcutaneous, and kidney knob fat) from seven Spanish breeds from meat and dairy aptitude, in kids slaughtered at 7 kg live weight was analyzed. There were differences between **breeds** on all depots studied, and the differences being more evident between aptitudes (meat and dairy). A dairy breed (Malagueña) showed higher percentage of monounsaturated (MUFA) and conjugated

linoleic (CLA) fatty acids than meat breeds. The highest percentages of saturated fatty acids appeared in meat breeds. Also, Horcada et al. [46] reported that it is possible to separate breeds and production system groups by discriminant analyses based on the differences between CLA and percentage of polyunsaturated (PUFA) fatty acids *n*-6 and *n*-3 and its ratio, for both intramuscular and subcutaneous fats. Also, dairy breeds are clearly separate from the other breeds by the composition of long chain PUFA fatty acids (especially C18:3 *n*-3, C22:5 *n*-3 and C22:6 *n*-3) on kidney knob fat.

With those results, Horcada et al. [46] concluded that breeds, together with the **production system** (understood as feed system, type and duration of suckling, type of milk), are important factors that influence and modify the fatty acid profile. It is convenient to remark that in young suckling small ruminants, the fat composition is closely related to the composition of the milk consumed (especially short chain and saturated fatty acids, SFA) [38, 46]. Therefore, the possibility of using the fat composition as a tool to discriminate breeds and fattening diets of goat kids has been proposed [46, 47].

Age and **slaughter weight** also modify some fatty acid groups on intramuscular fat depot. In Ref. [38], it was reported that fat composition of the meat aptitude breed Bermeya slaughtered at two different weights (7 kg CCW vs. 10.5 kg CCW) was statistically different on the percentage of PUFA, *n*-6, *n*-3, and its ratio. Heavy animals presented lower percentage of PUFA (14.16% vs. 21.49%), *n*-6 (9.03% vs. 14.31%), *n*-3 (4.46% vs. 6.54%), and PUFA/SFA (0.32% vs. 0.58%) with respect to those from light weights (younger animals). In the same study, a dairy breed was analyzed (Murciano-Granadina, 5.6 kg CCW), and presented a fatty acid profile more similar to lamb (10 kg CCW) than to Bermeya light kids. There were no differences in SFA between the **type of animals**, with values comprised between 46.5 and 42.3%; dairy breed had a higher percentage of MUFA (40.49%) than meat animals in both weights, similar to *n*-6 PUFA than heavy kids (10.23% and 8.39%), and lower *n*-3 (1.12% vs. 4.46%) and higher *n*-6/*n*-3 (7.79% vs. 2.26%).

Comparing kid and lamb fatty acid profile, the fatty acid profile from lamb differs more from meat goats than from dairy goats [47].

In conclusion, the intramuscular fat from suckling kids has an appropriate nutritional lipid index, and a moderate consumption may contribute to an overall balanced human diet.

6.5. Sensory analysis

Sensory data can be obtained from a *trained taste panel*, which assesses the sensory profile and the intensity of the evaluated attributes or by a consumer test where acceptability scores of the hedonic perception about the evaluated meat samples are obtained.

A trained taste panel used in [34] described goat sensory profile with the following attributes: kid and milk odor and six different flavors such as kid, fat, milk, metallic, acid, and bitter. Odor or flavor intensity between studied meat purpose **breeds** was not different. However, there were significant differences among breeds in the texture attributes, as tenderness, juiciness, and

fibrousness. Sensory attributes were highly correlated with each other, being the breeds considered as most tenders also those that had the highest values on juiciness and lower fibrousness values.

Also, no differences in flavor between different aptitude crossbreds have been reported in the other study [37], with a flavor intensity comprised between 6.3 and 6.1 on a 9-point scale, but there were differences in tenderness and juiciness. The most tender and juiciest breed (a crossbred from meat and wild aptitude) was the same breed that presented the lowest cooking losses and lowest shear forcé, as it was expected [37].

Differences between dairy and meat purpose kids have been reported on the intensity of species odor and flavor [38], which was slightly higher in dairy kids. Differences reported on tenderness and juiciness were affected by breed and **slaughter weight**. Kids from heavy meat purpose breeds had lower values on those variables than light kids, in which values were also similar to a dairy breed. There were no differences in other sensory variables such as fat odor, fibrousness, greasiness, or other flavors (metallic, fat, milk, acid, spicy).

Also, in [34], **slaughter weight** not only produced significant differences in kid and milk odors, but also in tenderness and juiciness. Applying a multivariate analysis, light kids presented a tenderer and juicier meat than heavy kids whose species and milk odors were more intense than in younger animals [34].

Then, slaughter weight has a stronger effect in organoleptic characteristics than breed per se, as happened in lamb, because the volatile precursors of aroma formation [48, 49] and their contribution to species flavor increases with animal age, although it could be modified by the diet [50].

The differences between light and heavy carcasses in fat odor intensities (higher for heavy) can be associated with differences in the amount of adipose tissue, which imports the distinctive aromas in lamb [51], because fat traps aromatic compounds and enhances taste [52].

Analyzing the effect of the **breed** on *consumer acceptability*, consumers reported differences between breeds on flavor acceptability, tenderness acceptability, and overall acceptability [16]. Kids from dairy breeds presented higher scores than other meat purpose breeds, which may be associated with the precociousness of fat deposition in these breeds and its highest fatness scores [16].

When different **slaughter weights** are compared [38], heavy kids present lower tenderness acceptability scores, without differences in flavor acceptability and being the overall acceptability similar between heavy and light kids of meat purpose. This indicates that in spite of several intrinsic differences between those light or heavy kids, in general, the consumer do not perceive the final product as different, despite coming from different weights, ages or production systems, with a suitable overall acceptability.

However, kids from dairy breeds had the highest values of acceptability both in tenderness and in overall respect to any other types (light or heavy), which show that dairy kids are preferred respect to those from meat breeds.

As it has been previously commented in the introduction, consumption of goat meat in Western countries is less common than lamb consumption, but there are no doubts about the specificity and own sensorial characteristics, attributes, and qualities of goat species. As Hungarian Sheep and Goat Breeders' Association have reported [20] "Goat meat is a very palatable food with several positive physiological effects and nutritional values. Generally we found in the market meat from young animals, mainly as a by-product of milk production." Animals are slaughtered young because consumers do not favor the strong taste and odor of older animals. Also the best meat yields are those from kids of 8–10 weeks old. Goat meat can be prepared in various ways, being the most usual fried or stuffed and roasted. Goat meat is slightly sweet, so it requires careful seasoning.

Comparing plain or seasoned products from goat or beef [53] showed that goat meat is always differentiated in a triangular test. However, acceptability scores are as high as those from beef, when goat products are served before beef products. It was hypothesized that the different order effect is affected by the familiarity or unfamiliarity that the consumer or panelist has with the product taste. When goat meat was served first, panelists had no comparison basis for their rating of the unfamiliar meat, and thus goat meat was scored better than when it served after a more familiar meat as beef.

In Ref. [54], it is shown how it is possible to differentiate and create a sensory map with 15 species by their own attributes. Excluding color, which was the main differentiating factor between species, the odor and flavor explained 66% of variation with texture representing 13%. Goat shows gamier, metallic and liver odor-flavor than lamb. Goat meat attributes were more related to beef and beaver meat than to lamb. In this sense, there are a few studies that compare kid and lamb meat, and results as those from [55, 56] reflect that lamb and goat differed mainly in aroma, tenderness, or fibrousness, but in very young animals, those differences decreases tending meat of both species to be similar. Nevertheless, [57] have not reported statistical differences in odor, juiciness or overall palatability between both species. And as reported in [16], consumers under different testing environments had some similar acceptability scores for lamb and kids.

7. Conclusions

In European countries, usually goats are slaughtered with a low weight being kid or suckling kid, the most demanded product, presenting carcasses that usually are lean, and having poor conformation and low subcutaneous fat.

In tissue composition, goat species present generally a high muscle and bone percentages and low fat contents. Perirenal fat is very important in goat, being also a remarkable criterion for carcass quality and classification.

Related to meat quality attributes, pH results usually are higher in goat with respect to lambs. Also, goat (kids) presented low color intensity (small values of luminosity, yellowness, Chroma and hue).

Usually tenderness is higher in lamb than in goats; The difference associated with differences in collagen content and other factors such as fat content or muscle fiber composition also affects tenderness. Small differences between breeds are reported only at short aging periods.

Related to proximate composition, meat from goat is characterized by a higher water content, lower energetic contribution, and lower fat content than ovine species. Being, on kids, possible discriminate breeds (purpose) and fattening diets by their fat composition.

Related to sensorial acceptability of meat from goat, there are differences between breeds and age of slaughter, being preferred young kids respect heavy or adults by presenting not so strong taste. Overall, milky to meat purpose kids mainly affected by a higher tenderness of milky kids are also preferred.

It is undeniable that goat meat has it own characteristics, which are different from other ruminant species; however, it presents a good acceptability comparable with lamb meat, a species whose consumption is more worldwide spread, especially when young and milk kids are considered; then, an adequate cultural development of goat meat characteristics could contribute to its consolidation by increasing its demand, because it has its own characteristics and singular high-quality attributes.

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Animal Health

Chapter 13

Parasitism in Goats: Husbandry Management, Range Management, Gut Immunity and Therapeutics

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Additional information is available at the end of the chapter

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Abstract

Goats play a vital role in the economy of common man. It acts as pivotal point in the uplift of socio-economic status of females. The goats are such delicate and fragile animals that encounter a lot of infectious and non-infectious diseases including viruses, bacteria and gastrointestinal parasites (GIP). The goat being a range animal is selective feeder. It needs a lot of managemental practices which safeguards its health. This chapter focuses on management, impact of gastrointestinal parasites, role of intestinal immunity, various breeds reared in Pakistan, role of plant based phytochemicals to treat against GIT parasites and various models to predict the status of health in animals.

Keywords: gastrointestinal parasites, gut immunity, goat management

1. Introduction

In Pakistan the economic losses due to worm infestation either internal or external worms like tape worm, round worms, flukes, ticks and lice are very high, although no exact figure is available. Millions of rupees are lost every year in the form of reduction in milk production, rejection of poor quality meat, depreciation of wool and hair, skin and hides, delayed puberty or breeding age, slow growth rate, death of young stock, high production cost and wastage of feed, less consumption of feed and poor digestibility. It is normal routine practice throughout

the entire world to control worm infestation to maintain the health status and production in livestock (cattle, buffalo, sheep, goat, camel and horses). In Pakistan, livestock productivity is low and genetic potential is not being fully exploited due to less development in Veterinary sciences or Veterinary biotechnology. Among all, unleashed worm infestation is the most important one. It is a ubiquitous phenomenon affecting all classes of livestock especially to the goats that is hampering the development of livestock/ goat industry in Pakistan. The worm infested animals become unthrifty, lethargy, less responsive leading to chronic diseases and ultimately death of young and older animals irrespective of breed, sex and age. The gastrointestinal tracts (GIT) of animals harbor a wide variety of worms named as nematodes, cestodes and trematodes collectively called as helminthes and few species of Protozoa and externally infested with verities of ticks, lice, mites, fleas and flies. The most prevalent nematodes or round worms are identified as species of parasites included Strongyloides, *Haemonchus contortus*, *Bunostomum phlebotomum*, Oesophagostomum spp., Cooperia spp., Trichostrongylus spp., *Toxocara vitulorum*, *Ostertagia ostertagi*, and Nematodirus spp., rumen worm and lung worm. In cestodes or tape worms are *Moniezia* spp. especially *Moniezia expansa* in small animals (sheep and goats). In trematode or flukes like liver fluke or *Fasciola hepatica* is notorious among small and large ruminants. Coccidiosis is a protozoan disease that can also infect small ruminants. Among protozoa the coccidiosis *E. arloingi, E. christenseni*, and *E. ovinoidalis* are highly pathogenic in kids. *E. ninakohlyakimovae* is the commonest one followed by *E. arloingi*, *E. caprina* and *E. hirci* with prevalence while others were recorded including *Eimeria parva, E. ahsata, E. faurei, E. caprovina, E. granulosa*, and *E. crandalis*. Clinical signs include diarrhea with or without mucus or blood, dehydration, emaciation, weakness, anorexia, and death. Cause enteritis and bloody diarrhea leading to less assimilation and causing anemia and weight loss of the animals other than Giardiasis and Cryptosporidiosis. External parasites like ticks, lice, mites, fleas and flies are not lagging behind to others. They equally cause losses, low productivity, blood drain/ losses, less concentration to feed and loss in milk letdown especially during dusk and dawn and even loss of life especially in exotic animals. By the timely and effective control of worms may uplift the socio-economic position of the female farmers of Southern Punjab and even by effective communication measures could result the saving of life and money from loss. The economic losses caused by Fascioliasis (*Fasciola hepatica* and *Fasciola gigantica*) results more than US$3 billion yearly loses in worldwide by mortality, reduced production of milk, meat and wool. Parasites, both internal and external, can drastically reduce the efficiency of an entire cattle herd. Only 5–10% of internal parasites actually reside within an individual animal, and the rest are present in the pasture, infecting the animal as it grazes. Knowledge of the developmental stages of those parasites outside the animal is key in making management decisions. The easiest and fastest decision to make regarding strategic parasite prevention is pasture management. While the warmer winters the past few years have contributed to keeping grass stands high, they have increased parasite risks in other ways, Mild winters increase parasites' ability to overwinter and multiply faster in the spring. Warmer temperatures lead to higher parasite risks. We always see a spring rise, if you have high parasites in the summer and starting now, you might need to think about altering your management. Burning pastures could be a significant management decision in eliminating a majority of the parasite load in the pasture, especially in the case of an operation with a spring burning program. Dewormer choice is also essential to developing a sound parasite

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management strategy. There are three common classes of dewormers: Benzimidazoles, or the familiar white oral dewormers, cover a wide spectrum of activity but offer no residual effects. Levamisole is only effective against adult worms and has no residual effects. Macrocyclic lactones, like ivermectin, have increased residual effects and retain high blood levels over a period of time. A combination of two has been found the most effective treatment, especially against resistant parasites. Using products that do not fully eradicate an animal's parasite load can also contribute to parasite resistance. The way resistance works is primarily genetic, you are selecting for a population of parasites that are resistant. Both internal and external parasites can develop a resistance to modern parasite prevention methods, Horn and stable flies have a large yearly economic impact in terms of efficiency lost and sickness caused in cattle. In just the U.S. cattle industry horn flies alone cost $1.36 billion in losses. Those include cost of control and the economic losses they incur for individual animals. Flies and other external parasites cause reduced weight gain and stress to animals. They also reduce efficiency, causing animals to require more feed to put on a pound of gain. Horn, stable and horse flies cause the bulk of external parasite problems. If an animal has an average of 270 flies on its body over the course of a day it loses up to 65 cc of blood. The economic threshold is currently 200 flies per animal and is a good indicator for when to begin fly treatment. Pour-on products are also effective but require a substantial amount of reapplication. We are running into resistance in all forms of parasite.

2. Materials and methods

The work was done for the record of parasites prevalent in Pakistan especially in goats. The data was obtained with latest tools available for collection and searching from web. The latest regime was also recommended for developing and underdeveloped countries to avoid any undue losses by any outbreak in goat population. All the resources were utilized to get the maximum benefits for the small ruminant farmers. The gut immunity was also explored which could protect the goat from infection(s) and parasitic infestations at GIT level. The contemporary data about ethno or phyto ingredients was also obtained and incorporated in the manuscript which was possible.

2.1. Effect of rangelands on the productivity of goats

Rangeland is an exclusive source of nutritious browse and graze species of plants in the regions where there is limited or no alternative feed resource available [1]. In arid and semi-arid regions of the world where there is scarcity of water and uncertainty of precipitation, the shortage of feed for small ruminants becomes eminent. So the grazers then look forward to range resource for grazing as their only option [2, 3]. Shrubs and trees which serve as browse species for goats and other ruminants tend to adapt harsher agro climatic conditions and at the same time are able to produce forage for longer periods of time. These species require little attention after its initial establishment and will be available for feeding when other feed resources are their lowest level [3]. Besides, number of natural, biological

and technical constraints, rangelands is still the major player when it comes to world food production. This can be understood when we see that rangelands all around the world is an exquisite food resource of about 1.2 billion cattle, one million sheep and 500 million goats [4]. Goats are considered to be the very first domesticated animals in the human made farms. It is noted in many archeological evidences that they have been assisting mankind and their farms for about 10,000 years [5]. There are about 861.9 million goats in the world. Among this huge number of goats, most of it is present in the continent Asia ranging about 59.7% out of the total number available in the world [6]. China remained on top in terms of goat availability followed by India, Pakistan and Bangladesh carrying almost half of worlds goat production load. Goats are usually kept because it is not much expensive to keep, because of the low space that it occupies as well as little feeding requirement and maintenance is far cheaper than a cow [7]. Goats all around the world are producing about 15.2 million metric tons of milk. India is the leading most in terms of goat milk production while Sudan and Bangladesh are behind India in the list. In Europe, Spain and France are leading producers of goat milk [6]. While the country that was most scrumptious in producing the goat meat was again from South East Asia i.e. china. The leading goat meat producing countries are from Asia and Africa. This shows that how important is goat's meat in these regions. Due to the increasing instability of different economies all over the world, livestock feeding is somehow experiencing serious setbacks in terms of qualitative and quantitative provisions of nutrients. It is happening all due to constant increasing of feedstuff prices in the market. So, the goats and sheep usually are exposed to low quality fibrous feeds such as stubbles and straws. These feed resources are very poor in terms of nutrition. So it can cause unbalance in the provision of complements that is actually necessary for the proper maintenance and production of goats and sheep. Researches have shown that when goats and other livestock are fed with concentrate feeds such as maize, barley and soybean meal etc. The growth of the animals is considerably increased [2, 8]. But these types of strategies require large amount of capital and expenses that smallholders are unable to cope. Here comes the role of rangelands in feeding these animals. As the rangelands are vast areas with plenty of grasses, trees, shrubs and herbs. And if managed properly they can be flooded with year round nutritious forages that are extremely palatable and loved by the goats [9]. In rangelands the type of grazing in which goats are involved, in most areas of goat abundance, are called as communal system in which goats and other ruminants are free to roam all over the field. And in the process they are grazing and browse variety of forage resources [10]. They do so by using their nutritional wisdom and in this process they most of the times are successful in balancing their diet order to fulfill the requirements of their nutrition [11]. Arranged two types of feeding patterns for goats in order to check there performance in both situations. He took a herd of goats divided them in two groups. Left one of them in rangelands grazing and browsing the range forage species. While the other groups was kept in farm where they were allowed to intensively grazed in small paddocks with feed supplementation and periodic treatment against gastrointestinal worms was done. The result after a specific period of time was taken out and when the growth rates were determined the results were of minimal difference, which means that small holders that are short of capital and investment can rear out goats in such regions [12]. Made a strong opinion that goats have special inherent characteristic of resistance from dehydration. Most breeds of goats have far more wider

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choice of vegetation as compared to any other ruminant. They also have a special love for browse species which make them more favorable in those rangelands where there is scanty and erratic rainfall. It is because of their wide palatability they tend to take out far more total digestible fibers from the plantation and due to this they have far more efficient digestibility of fiber as compared to sheep and cattle. So this shows that how any rangeland would be just perfect fit for small land holders in order to use either its meat or milk productivity. Various researchers across the globe presented their findings relative to rangeland vegetation and goat productivity [13]. Proved that goats fed in rangelands can give appropriate productivity especially for landless laborers or shepherds. He further added that due to relatively smaller size of the goat's body, limited water intake, low metabolic requirements and their ability to economize the nitrogen requirements make them an efficient converter. They become the best pick for surviving in the rangelands by giving their nurturer a considerable amount of meat and milk productivity [14]. As rangelands have vast areas consisting large variety of vegetation in it for the disposals of the goats, there are considerable chances that it could also feed on the toxic plants thereby inflicting serious biochemical changes and possibly could die. But according to [15] goats have a better ability to detoxify tannins than sheep. It is because of certain kind of bacteria present in its rumen that allow the detoxification of those toxic compounds. So those poor livestock farmers having less resources can take tannin containing forage which is cheap and easily available and mix it with small amount of concentrates can easily avoid periods of feed shortage in goats [16]. Goats have higher digestibility as compared to cattle and sheep, so that's why they are able to consume those woody species that are rich in tannin and could not be either grazed nor browsed by various small ruminants and large ruminants. It is due to the secretion of specific kind of saliva that has far more quantities of nitrogen as compared to sheep. So this becomes the founding stone of their enhanced meat and milk productivity using less nutritious feeds. Chemical composition of wheat stubble and other leftovers have shown that the energy content present in these feed sources are not that as nutritious as some of those shrubs and woody species. That's why the growth of the range fed goats sometime shows an increasing trend [17]. But in order to get an increased goat milk production, there should be a clear understanding of nutritional factors that could limit the milk production [18]. Mellado et al. [19] Depicted that goats in the rangelands of Mexico were allowed to feed specific shrub species such as creosote bush, prickly pear, and tarbush and side oats gram. The goats that had more fecal p level had low milk yield which showed that could not able to digest the nutrient thus cannot produce more milk. While those goats having low quantity of fecal p level had high yield of milk. This can be concluded by simply putting in a way that Rangeland fed goats will be a little lighter in weight. They may have a little lower average daily weight [11]. But still there performances in arid or semi-arid rangelands still cannot be overlooked especially for those landless laborers and small land holding farmers.

2.1.1. Goat management

2.1.1.1. Care of pregnant goat

Keep the pregnant animals separated from other ones in an isolated pen. Proper bedding material should be there for pregnant animals. Provide them adequate nutrition with easily

digestible and laxative feed along with fresh and ample clean water. Do not allow them to fight with each other and to mix with recently aborted animals. Clip hairs around the udder, hind quarters and tail for greater cleanliness. Dry the lactating pregnant doe at least 6–8 weeks before expected kidding. One attendant must be in near surrounding the pen for any emergency. The does must be assisted in case of dystocia. After the delivery some feed material with quick energy source should be provided to the doe [20].

2.1.1.2. Care of kids

Immediately clean the nostrils and confiscate the placental membranes, with the help of dry cotton. Clear the respiratory tract by hanging down the kid for few seconds. Allow the doe to lick the kids dry. Dip the terminal part of umbilical cord in tincture iodine to avoid the contamination at the interval of 12 hours. The newborn should fed on colostrum within 30 minutes of birth.

Following points should be kept in mind regarding the proper management of kid.

- Decontaminate the umbilical cord with tincture of iodine as early as possible.
- Keep the kids protected from extreme climatic conditions, predominantly during the first 2 months of age.
- Dehorning of kids within first 2 weeks of life.
- Deworm the kids at the age 2–3 weeks
- Vaccination of kids according to suggested schedule.
- Weaning of kids up to 8 weeks of age.

2.1.1.3. Care at milking

Lactating doe must be kept away from the buck. Normally milking should be performed twice a day. To avoid the injuries to the udder, milked the animals gently by thumb and first finger, followed by third finger.

2.1.1.4. Care of young doe

They should be provided with good quality feed and fodders. Stock for breeding purposes must be weighed and recorded on weekly basis. Prevent the young doe from inactions by adopting regular vaccination schedule as **Table 1**.

2.1.1.5. Management of doe

The estrous cycle of doe is 18–24 days on an average of 21 days. The heat period is of 2–3 days. The gestation period is 151 $\pm$ 3 days. Generally, the breeding season is spread all over the year and under good feeding and management conditions, two pregnancies in a year could be possible. Prolificacy rate of goat is very high; it can have from one to five kids. Twins are the most common birth rate. Four or five is very rare.

Sr. No.	Vaccine	Packing	Dose	Price	Month
1	CCPP	1000 ml vial	1 ml	PKR 18	May and Nov
2	Anthrax spore	300 ml	0.5 ml	PKR 402	Feb and Aug
3	Foot and mouth	300 ml	2.5 ml	PKR 454	Apr and Oct
4	Enterotoxaemia	300 ml	3 ml	PKR 67	Jan, June, Jul and Dec
5	Goat pox	1000 ml	1 ml	PKR 70	Mar and Sep

Table 1. Vaccination schedule of goat and prices in Pakistan.

2.1.1.6. Management of buck

Trim the hairs of buck from the prepuce region. Do hoof trimming of the bucks for proper functioning. Provide adequate amount of extra supplementation to the buck. Ensure the proper exercise of buck for achieving the better breeding efficiency [21].

2.1.1.7. Feeding management

Almost 70% of farm economics depends upon the cost of feeding. Goats have a high nutritional requirement. This requirement varies with stage of production, stage of growth and the type of production system. With the exception of milers, high quality browse and forage will meet mostly the goat's requirements. The intake of feed in goats is about 4.5% of the body weight, a higher value than other farm animals as they consume 4.5 pounds of dry matter per 100 pounds of body weight per day. Goat is a fastidious animal which is very selective for her feed, she is consuming one type of feed at a time may be not even interested in that at very next moment [22, 23]. Understanding the stages of maturity and how this affects forage quality is important.

2.1.1.8. Housing

Housing and its needs will base upon the production system. For fattening purpose an infrastructure that can protect the animals from seasonal extremes like heat, cold and rain is recommended. For a dairy purpose a separate place for milking parlor and to keep the kids isolated from their mothers. In range as well as confined feeding systems, the provision of housing is imperative. Shed must be built to provide shelter. Goats are afraid of rain and wetness which make them prone to pneumonia. Shed must be well ventilated, drained and should be easy to clean. Flooring should be provided and elevated at least 15° to facilitate drainage and cleaning. Separate pens should be provided for lactating, dry and pregnant does, kids and bucks. Required flooring space is 0.75–1.50 m^2 and feeder space is 15.24–25.40 cm for adult does and bucks. For growing goat the flooring [24] space is 0.50–0.75 m^2 while feeder space is 10.16–15.24 cm. 0.20–0.50 m^2 flooring space is for kids and 7.62–12.70 cm feeding space is for kids.

2.1.1.9. Management systems

Mostly there are three management systems practiced in Pakistan. First is intensive management system second is semi-intensive and third system in vogue is extensive management

system. Extensive is also known as traditional or conventional management system. Mostly the herders practice this system for goat raising. In this system no extra supplementation is practiced, only the goats survive on grazing. While some herders practice semi-intensive system in which they offer extra supplementation to the goats. The intensive management system is very rare in Pakistan, only few people practice this feedlot system in which they offer feed and water in tie system. Very few farmers available which practice this system. Mostly people offer extra supplementation in breeding season and in winter when there is shortage of food [25].

2.1.2. Mechanisms for immune regulation in gut in parasitic management

Goats are bowsers unlike other ruminants [26]. Their digestive framework makes them competent to except only plants that are not contaminated with their fellows with urine or feces. This provide them with very efficient system that protects them from ingestion of any parasite infestation [27]. Goat do not have, on the other hand, well developed acquired gastrointestinal immunity. This make them especially susceptible to nematodes for their whole life with clinical and subclinical infections [26]. The first barrier to protection against various diseases is characterized usually rapid, non-discriminating defense system – innate or non-specific immunity. Entry of any infectious agent; parasite, bacteria or virus, into animal body, make immune system sensitized resulting in functional changes. This is termed as acquired/adaptive or specific immune response [26, 27]. During this specific response, several proliferations and secretions of soluble factors are synthesized that restrict further incoming pathogen. From this activation a specific remembrance within the lymphocytes is produced that enable animal to provide enhanced protection in future invasion by same organism(s). This lymphocyte memory facilitates the immune system to respond rapidly with specificity in upcoming events [27, 28].

The immune systems both components, nonspecific and specific, is built on array of structures, cells and various secretions. The combination of these all provide effective protection surroundings within the animal [29, 30]. The synergistic effects by various substances produced and secreted by colonized microbes in gut also join hands to already task of eliminating harmful parasites. This normal microbiota provides sufficient resources in addition to above mentioned nonspecific or innate immunity. The subdued challenge at early age, moreover, enables them to fight more complex and difficult subsequent encounters of pathogens also. The same normal microbiota later also plays role in animal digestion and vitamin B synthesis [31].

In recent years, large body of literature, it is shown that presence of parasites/worms within immune competent animals basically suppress various components of immune system [32]. This results in much restrained immune response. This unperturbed immune reaction could be against various allergens, gut flora, and parasites present within the animal body [26]. It is also illustrious from other findings that sheep reproductive success and survival with parasite encumbrance are also affected besides all other abnormalities in immune system [33]. It has been well documented that with low Parasitic Fecal Egg Count (PFEC) relates to eosinophil count to infected sheep. This could be assessed with Arena test [34]. The higher number of

eosinophils suggest sensitization of cellular response to infecting larvae [35]. Previous and updated works on the topic suggest that eosinophilia during helminths infections plays a critical role in providing protective immunity [36, 37]. Results from various studies have shown that antigens of developmental stages in parasites are very important for early immunodiagnostics. Identification of immunodominant polypeptides and their immunogenicity could affect serodiagnosis of animals from field or slaughter house with their sensitivity and specificity [38]. Thus identification of an antigen from immature stages lay a cornerstone to development of amphistomosis serological detection kit for the future [39].

It is now without any doubt that T-helper type 1 (Th1) and T-helper type 2 (Th2) cells balance may lead to inappropriate inflammatory responses i.e. high Th1 (or Th17) and Th2 or vice versa [40]. It is also coined that immunoregulatory effects of parasitic worms are direct decedent of Th1 cells [41]. Whereas Th2 cells are organ specific and response to peritoneum, pleural cavity, and liver [29]. That's why, in helminthic infection, it brings about allergy and asthma in a positive imbalance to these cells (**Figure 1**).

These immune regulatory activities also increase diversification in the process of pathogenesis and then in pathology. A pattern has been recognized in matured dendritic cells that drives Foxp1, a specialized transcriptional factor, helps to develop Th3 (T-regulatory cells) – a X-linked autoimmunity allergic dysregulation syndrome (XLAAD). After the formation of Th3 cells, two mechanisms exist that foresee immune response per say to not to create needless aggressive immune response [42].This is being the "Old Friends" regulatory activation of dendritic cells that basically bystander suppressive mechanism [43, 44]. The other being adamantly work on self, gut contents and allergens in goats and sheep. This activation brings allergy, autoimmunity and

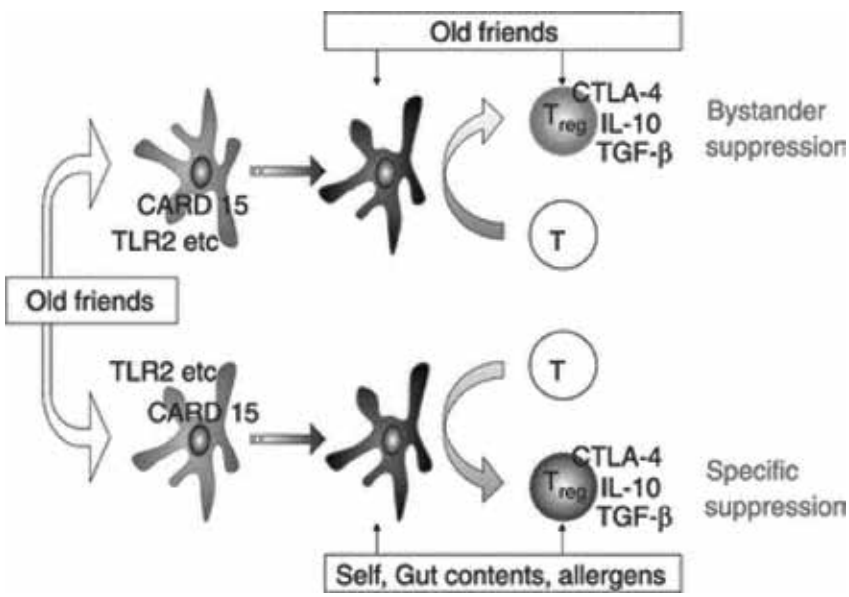

Figure 1. Immunoregulatory effects on parasitic worms.

enteropathy [40, 42]. The cellular component responding to the antigens or appears to act more vigorously in mothers than in neonates. Several cytokines specific to Th1 (IL-2, IL-12, and IFN-γ) and for Th2 cells (IL-5 and IL-10) are released into the system [45]. After the parasitic challenge, IL-2 has been estimated to be higher in neonates than their mother [56]. In the Th2 stimulation, however, IL-5 was higher in both mother and new born for intestinal helminths and filaria than bacterial sensitization [46]. Similarly, IL-10 levels were also similar [47]. After a concerted effect on the Th3 cells, they show crosstalk signaling that activated Mast cells, eosinophils and release of various cytokines [48, 49]. Presently, presence of early eosinophils is characterized with proinflammatory granulocyte that plays a key role in protection against parasitic invasion [36]. This creates a mainstay for the protective immune response to parasites as well as to allergic diseases [29]. The sensitized eosinophils produce cytotoxic cationic proteins including Major Basic Protein, Eosinophil peroxidase, and Eosinophil cationic protein [50, 51]. These proteins toil to work on both sides; protect from host prodigious infections and release of lipid mediators, oxygen metabolites and cytokines [50]. Presence of worm/parasite in sheep or goat may also trigger hyperactive immune response; reduced inflammation in the body leading to less severe autoimmune sensitization [52]. Recently, it has been demonstrated that (**Figure 2**) IL 5 is required for the blood and tissue eosinophilia in mice infected with parasites. Moreover, dendritic cells driven Th3 cells become sensitive to Myelin Basic Protein (MBP) that releases IL 10 and Transforming Growth Factor β (TGF β) [51]. Thus the presence of parasite enterprises to the autoantigen, resulting in inhibition of the process [28–42]. It is further demonstrated that parasitic infection depletes some important generalized and specialized transcriptional factors that are essential to transcription. One example of such factor is Signal transducer and Activator of Transcription 1α (STAT 1α), a key factor in IFN-γ signaling. Similar effects are exerted on epithelial C-C motif chemokine Ligand 20 (CCL20) – an anti-parasitic cytokine that is injurious to the parasite clearance [53]. In previous decades, number of factors, such as spring rise phenomenon, increased peristaltic movement were the focal point for parasitic infections. New interests are in line to enhance much appreciative strategies to control the parasite and worm

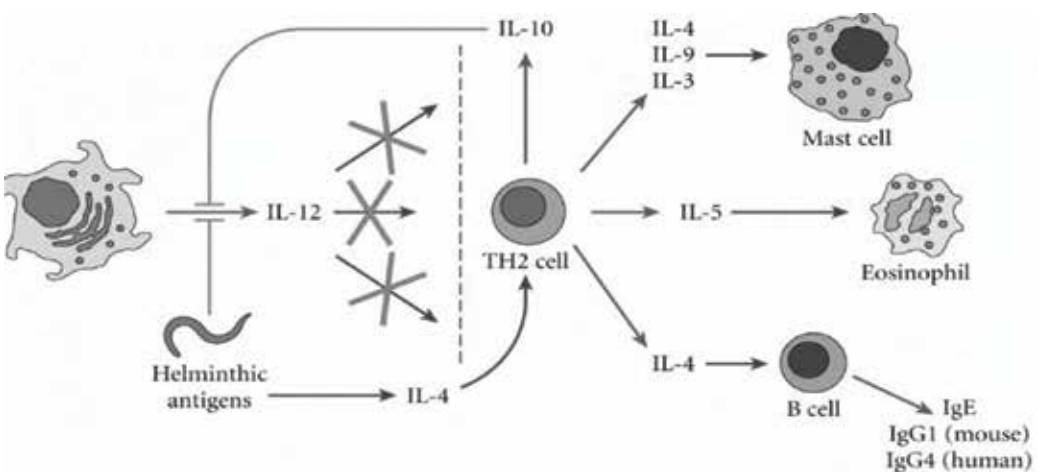

Figure 2. The cellular component responding to the antigens.

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infestations. Recent understanding on miRNA to both innate and acquired immune responses indicates its influential bearings [54, 55]. These small molecules (miRNAs) modulate immune system/cell differentiation, development, homeostasis and their functions [56]. Besides this, miRNAs also regulate a variety of developmental and physiological processes in parasites also [54]. These identities regulate effectively the development of Variant Surface Proteins (VSGs) that cover entire body of the parasite at one time and protect parasites for survival an attack by host immune sensitization [57]. Evolutionary studies on development of pathways for miRNA in parasites showed that they share different features than to other metazoan organisms [54]. This is perhaps, a finding that shed light on the miRNA induced silencing networks in the time frame of evolution [57]. In literature, a number of citations suggest that besides inflammatory response, circumventing several hundred gene products, parasites themselves can regulate their gene expression profile as well as produce effects on host genes [54]. Both these parasites and hosts encodes various miRNAs. Several miRNAs and miRNA clusters have been lined up to show specificities to T-cell helper (Th) lineages and functions. Role of miR-155 in protective responses to GI nematodes by both T and B cells is well elucidated [53, 58]. Conversely parasite on one hand make use of cellular environment for its survival and propagation (**Figure 3**). In return cellular pathways make this comfort zone unbearable/survival for the parasite [54–59]. During the parasite invasion, an alteration at host miRNA expression profile could drive parasite in infective mode or vice versa [57].Recently it has been documented that miRNAs, like miR-155, miR-146 and miR-223 control the acute inflammatory reaction after it recognizes pathogens through Toll-like receptors [57–59]. In trematodes, very high expression of miR-71a and miRNA-71b at various stages of development is one example [60]. Similar finding was gathered for cestodes but not for nematodes [61]. A special interest in *Cryptosporidium parvum*, a parasite that does not have RNA induced silencing mechanism, showed that it can reduce the expression of let-7 miRNA. In return they upregulates Toll Like Receptor 4 (TLR-4) [63]. The downregulation of let-7 involves promoter sites that is also use NFκB – p50. Other miRNAs are also active for

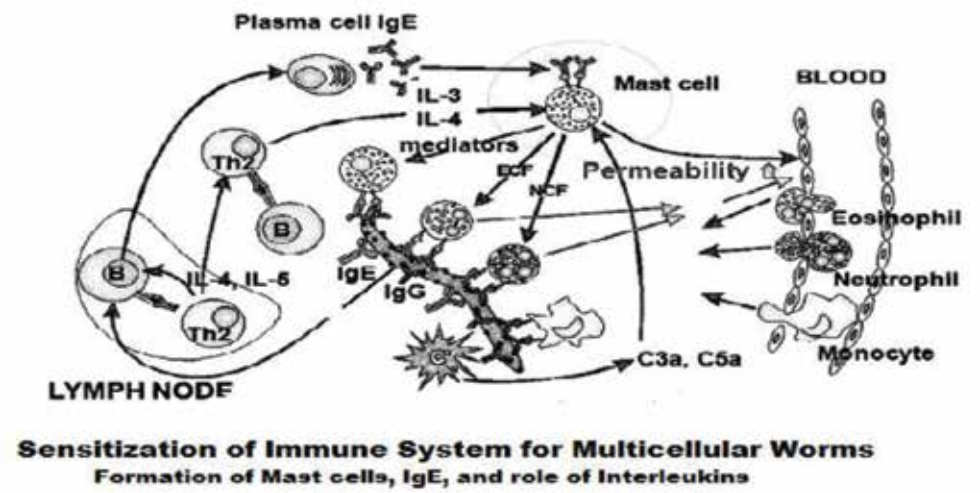

Figure 3. Immune response to multicellular worms.

NFκB – p65 in dependent and independent ways [62].The activation of these generalized transcriptional signaling leads to early epithelial responses. Consequently, mucosal immune effector cells (NK cells, dendritic cell, macrophages, CD4 and CD8 lymphocytes, and other innate lymphoid cells) invade the infection site resulting in sensitization to adaptive immunity [53]. In *C. parvum* it is shown that when miR-513 is repressed, it activation of B7-H1 expression on the surface which induces apoptosis of T-cells [68]. In *Trypanosoma brucei*, it has been suggestive that miRNA modulates expression of VSGs for the immune evasion by targeting 20S proteasome [54, 64].

2.2. Various plants and their activity on parasites of goat

2.2.1. Birdsfoot trefoil (Lotus corniculatus L.*)*

Birdsfoot trefoil is a perennial legume known for providing high quality forage with good yields on fields that have poor drainage or pH too low for alfalfa production. It yields less than alfalfa on optimal soils. BFT has a small seed size, as well as slow germination and seedling growth. Careful management is needed to allow for optimum germination and minimize competition from weeds or companion forages. BFT contains condensed tannins (CT), also called proanthocyanidins, which are naturally occurring plant compounds that significantly affect the nutritional value of forage by forming complexes with proteins, carbohydrates and minerals. Some proanthocyanidins are also detrimental to worms. Condensed tannins (CT), commonly termed as proanthocyanidins, are natural compounds originating from plants can significantly improve the nutritional value of fodder by making complex binding of proteins, minerals and carbohydrates. It has also anti-parasitic effects. Recently, Birdsfoot trefoil (*Lotus corniculatus* L.) has been recognized as auspicious CT forage, particularly for Northeast conditions. It can improve protein intake and is anti-parasitic in nature. Sanfoin (Onobrychis viciifolia) grass grown (western United States) and *Sericea lespedeza* (cold weather) are also identified as CT legume forages [65, 66].

2.2.2. Sericea lespedeza

Sericea lespedeza, a high-tannin forage (4–15% DM) has been proved to minimize parasitic burden in small ruminants. The mode of action is not yet clear but it has the properties to affect the parasites both directly or indirectly. Tannins may minimize the hatching of eggs in feces and arrest the developmental stages of larvae. It has the potential to bind with nutrients of feed and possibly block the bacterial growth that may act as source of nutrition for larvae. Tannins are helpful in reducing pasture contamination and animal worm burdens will help sheep and goats to be healthier and more productive. Previous studies have revealed that Sericea is highly efficient in controlling the endoparasites particularly when animals are grazed in open pastures or kept on dry food like hay or pellets [66–68].

2.2.3. Acacia karroo leaf meal

The nutrition value of fodders depends upon the stability of nutritive elements and digestibility of such nutrients, the metabolism and quantity of nutrients consumed by the animal.

High nutritive feed can promote increased level of growth and production. Tropical fodders like hay and straw have low level of nutrients especially nitrogen (N), in dry season. The contents of crude protein (CP) of such fodders (20–50 g CP/kg DM) does not fulfill the least requirement of crude protein (80 g CP/kg DM). *A. karroo* is documented two to three times richer in CP than grasses and cereal grains. The use of *Acacia karroo* as dry season protein supplements has been extensively reported in literature. *Acacia karroo* leaves contain high levels of CP and minerals. The CP values for *Acacia karroo* are within the optimal range of 120–230 g/kg DM required for body weight gain, maintenance and production requirements in growing goats. *Acacia karroo* leaves, also, have moderate levels of detergent fibers which are indication of high feeding values. The variation in the nutrient composition of *A. karroo* leaves observed and can be ascribed to differences in populations, soils, climate, and season, stage of growth, browsing pressure, assay methods and presence of secondary plant metabolites, respectively. Condensed tannins are the most common type of tannins found in forage legumes, trees and shrubs such as *Lotus corniculatus* and in several Acacia species. They are more copious in the parts of the plants which are more likely to be consumed by herbivores. There have been several notions regarding the basis for CTs synthesis which include protection against herbivory, plant defense against pathogens, nitrogen conservation, etc. The presence of CTs in *Acacia karroo* has been documented by several authors. *Acacia karroo* contains high levels of extracted CTs ranging from 55 to 110 g/kg DM. Consumption of tannin rich plant materials can be beneficial or detrimental to ruminants depending on how much is ingested among other factors. The negative nitrogen balance is as a result of complexation between tannins and endogenous proteins. Fecal excretion of N is a clear proof that CTs reduce the digestibility of feed. However, further research is needed to ascertain this observation. Browsing animals secrete proline-rich proteins (PRPs) which are considered to be the first line of defense against dietary tannins. The effects of proline-rich salivary protein as a first line of defense against CTs also merit further studies. Generally, there is dearth of information on the adverse effects of CTs in *A. karroo* on goat production [69].

2.2.4. Banana extract

The **banana extract** evaluated in concentration was well accepted by the animals, since the offered material was ingested by them, demonstrating having good palatability. In the trial period, the animals showed no diarrhea and changes in clinical parameters. The results strongly suggest that the ingestion of dried ground banana does not cause any deleterious effects evaluated the efficacy in vivo of banana leaf in the control of gastrointestinal worms in small ruminants, and observed no reduction of OPG in treated animals compared to the control group. According to the authors, this fact may be due to the short period of administration of banana leaves, or the restricted supply of 1 kg/animal/day. Assessing the anthelmintic efficacy of waste from the banana crop in gastrointestinal nematodes of sheep. In the *in vivo* test, the extract showed no efficacy, while the extract of leaves showed moderate efficacy. The authors suggest that low efficacy may be related to dose, extraction process or frequency of administration. The findings in these studies also report the absence of the anthelmintic action of banana extract in vivo tests, corroborating the results found in this study. In this study, there

was a significant reduction in hatching eggs Trichostrongylus, but was not observed for Haemonchus eggs. Natural products would be helpful to reduce the biochemical residues in fodders and forages of animal origin. The use of natural crops would further decrease the presence of chemical residues in foods of animal origin, mainly in sheep and goat. The diversity of the Brazilian flora allows for the possibility of utilizing various plant products to control parasitic diseases in livestock. A collective, systematic effort is necessary to incorporate functional or therapeutic foods into feed for small ruminants to control worm infections. Results suggest that *Musa* spp. has anthelmintic properties, as treatment completely inhibited *Trichostrongylus colubriformis* larval hatchability *in vitro* at two consecutive time points. The presence of tannins Musa spp. can promote the health of the animal. However, side effects are concentration dependent manner and extraction of these metabolites. Thus, studies are needed to define how to use, methods of extraction, analysis of secondary metabolites and dose in order to facilitate the use of these compounds in nematode control properties and, consequently, increase in the productivity of sheep industry. Therefore, bio panning of bioactive compounds and the development of an anthelmintic product containing condensed tannin would have great commercial potential [70]. **Table 2** is the already provided best anthelmintics for the control of parasites.

S. No.	Name of treatment	Classes	Description of classes
1.	Anthelmintics	Three primary classes of anthelmintics	**1.** Benzimidazoles (BZ) **2.** Imidazothiazoles/tetrahydropyrimidines (I/T) **3.** Avermectin/milbemycins (AM)
2.	Smart Drenching	Different approaches	**1.** FAMACHA **2.** Know the resistance status of the worms infecting the herd **3.** Keep resistant worms off the farm **4.** Administer the proper dose: **5.** Utilize host physiology to maximize drug availability and efficacy **6.** Split and repeat dosing
3.	Combination anthelmintics	Anthelmintics with other remedies	**1.** Vaccines **2.** Nutritional supplement **3.** Nematophagous fungi, **4.** Bioactive forages, **5.** Copper oxide wire particle boluses **6.** Various genetic approaches
4..	sound pasture management		**1.** Limit exposure to larvae **2.** Good management
5..	Novel non-chemical approaches	Copper boluses Predatory fungus Good management	**1.** Copper oxide with Haemonchus
6.	New drugs		**1.** Amino acetonitrile i.e., Monepantel-Zolvix

Table 2. Followings are the best treatment options of the parasitism in goat and sheep.

3. Conclusion

It is concluded that parasites pose a threat to goat. It is imperative to safeguard the goats and young kids what so ever the feeding/ grazing system is prevalent it must be vaccinated and dewormed regularly to avoid the helminths and protozoa prevalent in nature. The latest work which is aimed at search of phytochemicals which do not show any resistance against parasites in goat(s).

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Chapter 14

Complementary Medicine with High Dilutions Strengthen Conventional Therapies and Health

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Additional information is available at the end of the chapter

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Abstract

Breeders that have any concern to conventional therapies with sheeps and goats may be interested in complementary medicine with high dilutions. There are plenty of advantages in favor of adding these tools options to the animals care. Connections with breeders, animals, veterinarians and consumers trends are considered. The chapter discusses the context related to the opportunities the current state of art of high dilution medicines offers and the challenges that are faced by the mainstream current worldwide solutions. Six therapeutic styles are identified as useful for these small ruminants. Some of them need specialized professional support and some do not because they are already ready for acquisition and use. References of how work evolves in systems and how to find them are provided. The findings clearly state that the introduction of complementary high diluted medicines offers advantages to the current demands.

Keywords: ultra high dilutions, alternative treatments, residues, sheep and goats, small ruminants

1. Introduction

The contextualization of the usage of ultra-diluted medications benefits the understanding of the potential that this tool based evidences can provide operations with goats and sheep. The challenges and risks concerning the breeding of these small ruminants must be mitigated with an increase in the safety of this sector, and part of this optimization is the certainty of availability of non-residual therapeutic tools for veterinarians and full access to producers. Thus, ultra-diluted drugs may reduce the sanitary and management risks of the activity itself and provide a good animal product, entailing less tasks for workers who daily handle animals by decreasing the demand for work. Broadening the capability of maintaining the herd

healthy or making decisions in the face of an outbreak in a herd is a preventive measure, and, as is known about preventive measures, they are less costly than implementing a reactive remedial action in a specific disease or adversity.

In addition, the customers that eat and make use of animal products are becoming more demanding and are aware of their own health, especially referred to the risk of chemical, microbiological, and systemic contaminations of products and substances that will be exposed to the social-environmental impacts caused by the sector, which invariably can affect them. However, there are restrictions to traditional and registered medications liable to be used in goats and sheep, which aggravate due to the fact that innumerous classic and conventional therapeutic tools like antimicrobials and antiparasitic are at risk of functional collapse. Therefore, ultra-diluted medications may, individually or collectively, make the systems more competitive, reactivate in practice the healing capability of conventional treatments in a joint action, decrease the mortality of animals in outbreaks, and prevent an end of the feasibility of production systems that make use of goats and sheep anywhere.

2. Literature review

It is important to conceptually distinguish ultra-diluted to phytotherapic medicines regarding the occurrence of residual risks. Phytotherapic medicines are herbal drugs that generally have a smaller industrial processing and are based on the presence of concentration of chemical compounds present in parts of plants that are used. Like, for example, a passion fruit extract (*Passiflora sp*.) that produces a soothing effect if ingested, marigold extract (*Calendula oficinalis*) as a topical anti-inflammatory that acts in the region it has been applied, and garlic (*Allium sativum*) as antibacterial and antioxidant [1, 2].

On the other hand, the ultra-diluted medications can originate from plants, minerals, or animals, like, for instance, *Atropa belladonna* (dynamized belladonna) for acute inflammations, including some acute mastitis, *Natrum muriaticum* (dynamized NaCl) for physical illness due to separation at weaning; and *Apis mellifica* (dynamized bee) for acute allergic reactions. The dynamization process extracts from the original compounds the medicinal principle through consecutive dilutions and shaking. The original compound needed to make an ultra-diluted medication is called mother tincture and depending on the substance, it can be directly used as an herbal medicine before the dynamization. The debate on the active principles of ultra-diluted substances is important for the differentiation regarding the presence of residues, while herbal medicines and mother tinctures have high concentrations that can be excreted and modify the taste of milk and meat, and it might need a waiting period and disposal of milk, for example, ultra-diluted drugs have a low concentration of molecules and the risk of the presence of residues and need of waiting period or disposal derived from the medication is unnecessary. The presence of herbal medicine residues may be less significant than antimicrobials, hormones, and anti-inflammatories, but the local bodies that regulate drugs for animals that process food imposes legal restrictions even if not always complied [3, 4].

The results of the use of ultra-diluted medicines were adapted from human medicine and are independent of beliefs or religions, and the achieved effects are different than the placebo effect, even if animals or caretakers are not aware of the introduction of those medications. Generally, the healing processes in animals are remarkably higher in terms of qualitative response and time in relation to if they were compared to humans. Recently, a change in the research epicenter regarding ultra dilutions has been observed from Europe to Brazil. Example countries that are well-known for using ultra-diluted treatments are Brazil, India, and France, but there are veterinarians and doctors that employ this kind of treatment all over the world, and it can be easily noticed in some specific events of the sector, and it is clear that there is availability, and the access to such professionals will depend on a matter of choice. China has started a remarkable movement of research funding, whereas, against history, a group in the European Community has decided to impose a restriction to investments in research. In Switzerland, there are institutions engaged in research and cancer treatments with ultra-diluted and anthroposophical medications (e.g. Weleda, the Ischia Institute); in Italy, there are wards in hospitals that make use of very common ultra-diluted medicines in Toscana region. In Brazil, there are pharmaceutical industries of ultra-diluted medicines for animals that are distributed to retail and packed in sacks with up to 20 kg, which are shipped throughout the country and for exportation. The recognized benefits by popular use of people in charge of animals or caretakers in farms, or even in pets, as well as by a specific category of professionals impose to the agility and interest with which universities and public research centers are unable to get updated and make experiments. Thus, attention of opinions from individuals with a lack of deep area knowledge must be paid with restrictions. There is a need to check the origin of information and their reliability should be checked if comes from an ultra-diluted substances expert [5–7].

3. Material and methods

The following sections will identify the use of high dilutions with their opportunities and challenges.

3.1. Opportunities

Treatment of animals is part of the food supply chain for people, thus there is an interface with human health. People who choose ultra-diluted medicines are generally constituted of more educated and wealthier individuals, and it is known that the offer of this kind of therapy reduces the financial expenses of its users with healthcare plans. On the other hand, there is still part of the population that demonstrate problems with the use of conventional medicines (allergies, intolerance, adverse symptoms, recurrence of symptoms, and resistance) and that benefit from treatments with ultra-diluted drugs. There is an applied area of pharmacological studies that makes major efforts in the application of individualization and customization of treatments that employ therapeutic drugs, but are restricted to the enhancement of the manipulation of dose protocols or the introduction of a conventional medicine for the metabolism and predetermined conditions.

As well it happens for human beings, there is a population in the different species of animals that benefit from diverse degreed of restrictions to the mass conventional treatment protocols. Those animals produce less, suffer repeat infections, recurrence of symptoms, constant sickness due to different diseases, all of them generally caused by unsuitability to the proposed system in which they are classified. An animal that does not adapt may be disposed or transferred to another place in case it does not die before, but curiously the rate of adaptation in the herd returns to the previous level with a brief period of time and appears to be constant, regardless the disposal or removal of animals. Therefore, collective factors, either intra or extraindividual, may be present and are challenging study objects and still unknown either for animals or humans. Although with efforts being made in the customization of conventional therapies and its prominent proposals as a tendency in pharmacology, especially in humans, the use of ultra dilutions has already preceded that individualization in the west over 200 years ago.

Ultra-diluted medicines are versatile because they can be applied in many production models: since a conventional producer, who makes use of them as a first modification of assistance and support in handling; introduced in breeding to reduce costs with medications; activities for workforce and disposals; until producers with extreme needs in reducing feedstocks and residues that they incisively use; and learning and counting on specialized workforce to practically solve all their demands. Examples of systems like that are organic certified (bio), anthroposophical, independent systems of self-sufficiency, farms that produce food for a group of consumers that demand less residues in animal products and countries that were subject to trade embargos extended by the supply of feedstocks (Cuba), distant regions with reduction or geographical restriction of trade routes and even regions of social and economic exclusion.

The active principle and the mechanism of action are one of the most controversial issues, and the further development of the discussion involving them, due to the limitation of access to data, may be used or inadvertently prevent the access to the benefits that it entails when it comes to its practical and followed use favoring to the animals. The state of the art is that the effects in living creatures, including animals, were corroborated as a natural phenomenon and is at a stage of collection of experimental data to supplement a single theoretical and solid proposal. There are two dozens of theoretical propositions to explain part of the collected phenomena, but that depend on enhancement to create a more comprehensive theory that is capable of approaching the full extent and theoretical predictability of the analyzed phenomena. It is estimated that there is an interaction of the informed field effects (including the electromagnetic ones) with living beings, although some of the imposed obstacles remain in the own limitation of the knowledge about living creatures as biosensors for the fields. The hard core scientific developed knowledge on the interaction of fields with living creatures nowadays is restricted to an experimental and diagnostic effects, with X-rays, computed tomography, MRI scan and ultrasonography.

Even though all the accumulated knowledge so far allows the use as a therapeutic tool, taking into account, yet, that some types may rely on specialized work force to carry on and follow the treatments successfully. Possibly the medication acts informationally through weak

waves, probably and even electromagnetic (because there are waves different than the electromagnetic) that can be inactivated through the exposure of a few minutes at a temperature above 60°C. The medications that are carefully produced and properly stored are capable of influencing the health-disease processes of the animals that are biosensors regarding the interventions that are carried out.

Given the characteristics of the medicines, there is a reduction in environmental pollution due to the lack of metabolites and known drugs excreted in the milk, urine and feces (e.g. use of hormones to synchronize the mounting season, ivermectin as antiparasitics, antibiotics and anti-inflammatories in case of infections). The water treatment systems for human consumption and the waste treatment of animals are generally developed without the monitoring of the depollution by hormones and other drugs. According to the proposal of the productive system (organic example), the use of ultra dilutions becomes one of the main tools in the control of diseases, since there is a restriction in the use of conventional medicines, which is conditioning for the maintenance of the status of the proposed productive system. Thus, in such systems, we can consider that this therapy would have a more important function than the usual denomination that it has nowadays as "complementary".

The scope of the use of ultra-diluted medicines is wide and has a high plasticity according to the demand of who required it. The therapeutic tools are initially recognized or reached to the animals as a simple substation of conventional feedstocks. The progress of the dialog with the increase of interaction with a specialized professional and the broadening of the comprehension of the potential regarding the tool may coevolve until the complete substitution of conventional medicines in most of the cases. That myriad will depend on the personal investment made by people in charge of the animals and direction of the productive system, and there is generally need for identification of a distribution channel of animal products aiming at the selection of people who make a point of having those characteristics. An example of target audience are people who are identified with the use of items originated from family agriculture, organic products, anthroposophical goods and also regions in which there is social and economic exclusion with a restriction of access for the animals to medicines, routine laboratory tests for following-up the herd and eventually low investment in cutting-edge technology.

Examples of the use of ultra-diluted medicines in herds of goats and sheep are generally associated to the main demands, like, for example, it is notorious the presence of intestinal endoparasites, reduction of stress through feedlot handling, reduction of the impacts caused by Caprine Arthritis Encephalitis or lymphadenitis. Let us take a look at these cases:

Two findings revealed that the spontaneous growing curve of endoparasite eggs by natural infection are statistically lower (two-way ANOVA; P-value <0.01; $n = 7$ or 8) in pregnant goats treated with commercial medicines for internal parasitosis in comparison with water-treated animals. During the 10 weeks of intensive monitoring, the curve outlined by the result of the fecal egg counting test throughout the weeks maintains a parallelism in the elevation pattern among the groups. The animals presented a prevalence of Alpine breed with approximately 10% of the Saanen breed, part of the time in sheepfolds with elevated slatted and loose in the pasture in the hottest hours of the day.

However, the previous round of treatment phase was, mostly during the mounting season, the group that was subject to verum treatment also presented a lower monitoring for fecal egg counting, but that decrease was not significant in relation to the placebo treatment.

The monitoring was promoted by the importance of internal parasitosis in goats and sheep and the offer of commercial medicines produced and provided in Brazil, which are specific for the control of internal parasitosis. The animals, individually labeled and distributed by lots in randomized blocks, were given powdered medicine dissolved in water directly to its mouths in order to obtain more control in the experiment, and the control group was subject to the administration of pure water to mimic the restraining of each animal. Feeding consisted of feed and corn silage. A factor that increased the challenge of natural infection, especially in the treated group, was the disposal of animals from both groups in the same space and at the same time, that is, if all animals were treated the expected parasite load of the entire environment would be certainly lower. However, the natural infection in different pickets may be diverse, therefore the collective use for both groups can be considered as a smaller potential distortion of the results in comparison to the situation in which the groups were in different pickets. The main results were that the medication showed its action, statistically meaningful, during the supposed challenge due to the metabolic stress caused by lactation [8, 9].

There are reports of cost reduction with total replacement of conventional medicines, even if considering the investments specialized in work force. This therapeutic tool has its own evaluation systems of the evolution of health-disease processes. According to the evolution of the state of art that identifies the interaction between parasites and hosts, it has been observed that immunological and metabolic variations regarding the modulation phenomenon, even subtle, may significantly change the detrimental effects of parasites on hosts, and they are specially interesting when such effects are minimized and when the capability of the animals to be kept productive is increased, making the system more solid to interferences and adversities.

Knowing how to observe the action time of ultra-diluted medicines is crucial to understand and properly evaluate its functioning. As a general rule, if a process is acute, that is, if it started suddenly or very quickly, the process and healing time will be immediate or very fast, like, for instance, in an acute mastitis, an allergic reaction to an insect bite, acute torsion of a digestive organ or problems in calving. On the other hand, if a chronicle process that has been taking place for more time, even if not previously identified by people in charge, might have its resolution time or higher balance, like, for example, caprine arthritis encephalitis, lymphadenitis and mycoplasmosis. Sometimes the synergic effect of a conventional treatment and a treatment based on ultra dilutions may gradually reduce the need of interventions, like the incidence of mastitis, myiasis and other ectoparasites.

Therefore, the peak risk of incidence of acute mastitis may be kept, but gradually reduces its intensity and number of affected animals according to the evolution of a continuous treatment, similarly with the occurrence of external and internal parasites. In a conventional system, the employee in charge of the animals that records the application of antimicrobials in cases of chronicle mastitis that become acute, and ivermectin to control the evolution of larvae that might affect the production of leather and wool, and of internal parasites in case he or she

monitors the mucosa through its color against anemia, he or she will notice that the interval of ministration of conventional medicines gradually increases in up to 3 months, drawing its own conclusions. The erroneous allegation by the false feeling that the proposed treatments fail is common when there is lack of records, and generally those progressive alterations slip, thus all that must be done it to check the records.

Milk-producing systems that need interventions for mastitis are subject to a period of initial adaptation. The ultra-diluted tools will stimulate the removal of possible pathogens present in the udder, and this phenomenon is known as drainage with exoneration. After the first days of the start of the ministration of the medicine, lumps in the milking will be observed, and it looks like to be acute mastitis, but what is really happening is the removal of agents with direct stimulus in the animal through the mammary gland. The difference between this phenomenon and an acute mastitis is that the animal quantitatively maintains the production of milk in a very similar way, does not present swelling nor redness in the mammal glands. Its feed intake and rumination remain normal, there is no fever, the animal moves normally and appears to be well and calmer during the milking.

Thus, the treatment with antimicrobials is dispensable and the use of intramammary enzymes is advisable in order to help to dissolve the lumps and remove them. This case is the only moment in which it happens and, due to the increase in the number of somatic cells, the breeder may choose for redirecting the use of that milk if it is paid due to its quality, according to the adopted criteria. The beginning of the treatment does not impede the incidence of clinical cases of mastitis, which will be typical, and the opposite of the parameters previously mentioned, except that it may also be included the presence of lumps or absence of milk production. The use of ultra-diluted drugs does not dismiss necessary handling ordinary care, like pre- and post-dipping, hygiene of the animals, of the milker and of the facilities, as well as its adjustment and recommended maintenance.

3.2. Challenges

Considering that the resistance phenomenon to antiparasitics and antimicrobials has been observed and reported more frequently than the speed of production of new drugs for animals, there is an imminent risk of the existence of a crisis in the conventional treatment tools. That crisis may have evolved either through the technically recommended use or specially through the indiscriminate use of active principles in some places. Curiously, it may have been preserved in places which it is not so used due to the restriction to access because of many reasons. Breeding with research and development, execution of practical tests, optimization, record and approval by regulatory organizations from each country or block for a new drug may take 20 years. That investment is high and the private sector eventually restricts the production and research according to the obtained financial return, that is, the offer of medicines may be influenced by adverse factors that go beyond the demand and needs regarding the animals, especially concerning highly technical medicines and with a limited or restricted distribution flow in the market [10, 11].

The expected use of medicines itself already imposes a risk of the active principle to lose its activity after a few decades, and this is aggravated by preventive and indiscriminate use, like

subdoses, lack of execution of antibiogram, duration and frequency lower than the recommended according to the pharmacological basis.

In accordance with the drug, like, for example, an antimicrobial, the phenomenon may involve the contamination of people with resistant microorganism through the intake of raw animal products, poorly cooked, non-pasteurized or without a proper inactivation of possible pathogens, and such risk is aggravated in human population with comorbidities, reduced immunological response and at risk, like for children, elderly, pregnant women, HIV carriers, people with transplanted organs and people who are subject to other continuous treatments by immunosuppressants.

For example, albendazole is a trend-setting antiparasitic for the treatment of round internal parasites either in animals or in humans, but, through time, the antibiotics ivermectin had to be used as an antiparasitic in animals and nowadays is employed in humans. The recommended cut point of the fecal egg counting test to start a antiparasitic treatment was broadened, and this increase may make the maintenance of the usage of albendazole viable. Several reports show the use of ivermectin with power of influencing and modifying in an undesirable way the gene expression. Its effects in the long term in an animal, for human beings who consume milk and meat from those animals, rest unknown.

Goats and sheep are domestic animals that moreover contribute for people's nutrition all over the world, therefore they are part of the trophic food chain of human beings. However, mankind nowadays suffers from an outbreak of cancer, even though too little is known about what reasons led to that increase in the sudden incidence of cancer in the last few years. It is speculated that factors regarding industrialization, environmental and nutritional pollutants may have strong influence, and curiously all of them are connected to the animal food supply. The most substantial data discovered was the identification of the consumption of cow's milk. This habit was pointed out as one of the identified risk factors that contributed to the higher incidence of cancer when comparing the Asian human population who did not consume milk.

The protective factor had that possibility due to the gene pool, but was disregarded after the confirmation that the incidence equates when a migration of that Asian population happens to Western regions where they had got into the habit of consuming milk and dairy products. This little-known fact is overlooked by professionals responsible for health, feeding and handling, as well as by animal breeders. Thus, feeding and the use of medicines for animals have a low validation of effects in the long term, either in the animals itself or in people that consume animal products.

The diet and the investment in animal's feeding is a factor that exerts a great financial impact in this activity, and one may add a great range of ingredients in animal's feeding without obtaining a validation of its effects, except the sieve of the animal itself in refusing to eat. There is also the possibility that the ingested feed by the animal cause an expected alteration in flavor/taste and strange odor in meat or in the milk for the person who will consume it (e.g. excessive use of sugar cane). Frequently the inclusion of those ingredients is reduced until is accepted by the animal to make use of the cheap energy or fibers source.

The manufactured medicines either for people's use or animals are subject to tests in the short term before they reach the market, even if there are different regulatory agencies according to the place. But, the tests in the long term in the so-called phase 4 are directly carried out in the population that make use of them and followed by the pharmaceutical industry itself, so there is a clash of interests regarding the demonstration of the results. The animal pharmaceutical industry does neither monitor nor follow the effects of the medicines used in animals in persons. Any kind of tests and experiments has a very high financial investment to compensate even the return with the sale of drugs. Therefore, we are facing a crisis not only financially, but also that concerns principles regarding the influence in feeding and people's health, which can be influenced by the animal handling. That contrast is shown when we identify a human health crisis through a cancer epidemic as the apex of a great problem, and, at the same time, we overlook time and effort granted to treatments and its effect in the long term in animals that provide food and supplies for people. This theoretical ideal may be very distant from the average practice in the world, especially because not even the access to laboratory tests for animals, medicines in sufficient amount and demand, either physically or financially, besides the proper disposal of animal products, are widely restricted in many regions and less developed countries.

4. Key results

Relevant therapeutic strategies qualitatively identified to small ruminants will be presented as the key results.

4.1. Herd treatment with predefined protocols

The simplest therapeutic strategies of immediate application, with low need of intervention of specialized work force, direct acquisition and generally without prescription, which contemplate the main solutions for caprine and ovine breeders were inspired in the theoretical utilization and practice of drainers (Léon Vanier) and biotherapeutics (Constantine Hering and Wilhelm Lux), developed, conceived and recently consolidated in Brazil. This new field is called, in veterinary medicine, as populational homeopathy and was designated by its creator, the Brazilian veterinary Cláudio Martins Real (1926-). The medicines can be administered to animals twice or three times a day in general, directly on the feed trough, at the customary feeding time, or mixed in the feed, or even in mineral salt. They can be administered directly in the animal's mouth in case there are a few animals or impossibility of including the necessary dosage in the intake routine.

Let us take a look at some examples:

- Control of verminosis in goats and sheeps: a general recommendation for all producers is to administer from 1 to 10 g of the commercial product feed trough, a daily dosage of 2–5 g per head is a customary recommendation. The beginning of a typical treatment starts with a higher dosage and, after 2–3 months, it can be gradually reduced. A high potential challenging situation can start with a total of 10 g per head, during the day, throughout 2–3 months, and slowly reduce until a cost-benefit of the desired effect is stabilized. The

aforementioned experiment made use of individual administration outside the recommended protocol of the package insert because, even with the dosage of 2 g per animal a day, the frequency had to be modified for a daily oral administration, for 6 days a week, always skipping Sunday. Commercial medications that are associated to this solution for internal parasites: Verm 100, OvinoSigo, Fator Vermes® [12–14].

- Hoof problems: commercial medications associated with Homeopathy for Herd of the veterinary medicine that may be used for caprine and ovine with official record of the benchmark Brazilian industries: Foot 100, CascSigo, Fator Casco®, Fator Diartro® [12–14].
- Mastitis: Mast 100, Mastisigo, Fator M&P Pó®, Dolisovet® intramammaire, PVB PHYTOLAC SOLUTION BUVABLE [12–15].
- Diarrhea: Entero 100, EnteroSigo, Fator Infecções Pó® [12–14].
- Reproductive system: Pró-Cio, Matrimax, Vigotonus, Embrioplus, FertSigo, SodoSigo, Fator Fértil Pó®; Fator Parto Pó®; Fator Cria Pó®; WOMBYL GA BUVABLE; PVB POUDRE CALCIQUE GA [12–15].
- Photosensitivity and skin: Figotonus, DermaSigo, Fator Hepa-Foto®, PVB ABCES GA BUVABLE [12–15].
- Feed conversion and fattening: Convert H, Convert H Leite, EngordSigo Plus, Fator Pró Pó® [12–14].

The usage of this kind of ultra-diluted medications [12–14] is constant during a period of time, and, in this case, the most widely used explanation and easiest to understand is that, given the challenges that are always present in the system, for example, restraining, even partial, pressure regarding the production demand, higher animal density, dependence of human interference for feeding, water supply and sheltering concerning free wild life. The role of the medication could be explained, according to this theory proposed by the creator of the method in 1987, as a continuous counterbalance of those challenges that are imposed to each organization, that is, the reason why there is a general recommendation of 2 g per head daily, which in practice works very well and that may be suited to each situation. It will be possible to notice improvements in the reduction of the treated symptoms in up to 3 months.

The specific sporadic usage to act in phases of the productive system, for example, promotion of ovulation and embryo fixation, diarrhea in young animals, skin and metabolic temporary problems of the liver. For medications whose goal, for example, is to control internal parasites, mastitis, if applicable, and to minimize stress and increase the utilization of feeding, it is advisable to continuously use 2 g per animal a day (or even 1 g) than interrupting this therapeutic type. The progress of the time of use monthly and annually will show to the person in charge of the animals that they are progressively better, more resistant to the adversities caused directly or indirectly by the treatment modalities.

It is important to highlight that the reduction of endoparasitism will not prevent the need of usage of an antiparasitic like albendazole because the medication is not supposed to completely

substitute the input, but rather increase the application interval gradually, increase the resistance capability of the animals to the effects of internal parasites, increase the challenge for internal parasites to remain alive and decrease the feasibility of reproductive ways of internal parasites. In the beginning of the rainy season and in the beginning of dry season, it is strategic to double the dosage for 1–2 weeks. This therapeutic modality depends on the dosage, therefore, when you notice that the challenged has increased it is strategic to increase the dosage per head, daily in two to three times. The higher the number of daily administrations the better; two are enough and in only one the assurance of consumption must be guaranteed, because there can be a lot of variation in the individual ingestion due to competition in the feed trough. The recommended availability of the size of the feed trough must be properly assured to the number of animals. For the daily total dosage, it can be considered the several ways of estimated administration of consumption orally, like addition in the feed, mineral salts and directly in the feed trough on the silage or feed. The powdered feed mixed specifically in the farm can be added in percentage value to the consumption according to the administration. Some pelletized feeds may contain oiliness enough to adhere to the means of the most used medicines (calcium carbonate or crystal sugar), however the inclusion of the formula before the pelletization is not recommended because it will certainly cause a loss of action due to the heating process higher than 60°C in the extruder.

The storage of the pure medicine, or of those that are mixed with mineral salt or feeding must be protected from higher temperatures (above 25–30°C). Sealed rooms without ventilation, vehicle or dumpster interiors exposed to the sun, proximity of strong electromagnetic fields may cause interferences, and these are easy to be avoided, because they are highly suspicious in terms of medicine inactivation. The availability in the mineral trough of exposed mineral salt to the sun does not prevent the functioning, but after the incidence of some rain washing it will have to be reoffered. After long periods of utilization, rarely the treatment effects may not be observed as if it had stopped, in these cases all you have to do is to suspend the medication for at least 2 weeks, use another drug, like, for example, instead of controlling internal parasites monitor stress, and then reintroduce the previous medication that had stopped working. These principles are applicable to all medicines of continuous use of populational homeopathy and the package insert may present different recommendations with each other, but all of them must work this way.

4.2. Self-organization Factor of the Biofield

The BioFAO methodology for animals – Self-organization Factor of the Biofield – was also developed in Brazil and evolved from the work of experts in human beings in a daily clinical practice by a doctor who worked in the beginning of her career as a unicist – Míria de Amorim. The application of this method in animals presents a differential among other therapeutic options with ultra dilutions, because it generally makes use of an application protocol of single day administration and with long intervals without the need of new intervention after the starting months of follow-up. As well as with other methods, it can be used in goats and sheep for breeding and pets, individually or collectively, but it demands the monitoring of a qualified professional. An updated list of qualified professionals is available [16].

This therapeutic tool used seven dynamized minerals in a specific sequence of medicines: *Antimonium crudum*, *Kali carbonicum*, *Mercurius solubilis*, *Sulfur*, *Natrum muriaticum*, *Aurum metallicum and Ammonium muriaticum*. The establishment of those medications was inspired and has a connection with the ayurvedic medicine used in India. This protocol is capable of producing in the animals the phenomena described in Hering's Law of Cure, that is, they trigger a healing process individually. Due to the fact of the usage only of ultra-diluted medicines, it presents the same benefits with minimum contamination risks of animal products previously described. However, it is necessary to perform a professional follow-up to determine the proper potency to animals or to the herd, monitor the occurrence of symptoms, guide the person in charge in case of need regarding the conducts that are possibly needed, frequently re-evaluate the status of the animals and make news prescriptions according to the collected data.

The advantage of this method is that is dismisses the need of repertorization, which is done by traditional treatments with ultra dilutions. Therefore, the prescriber decides especially about the potency to be used and the necessary protocol repetitions, which can vary according to the purpose of the activity with animals, the age of each category, historic, risk of exposal to interference and evolution agents. Another great advantage of this methodology is the administration of the medication, because the protocol is done in one single day for all animals. The medication causes in the animals the cure process that will act in the subsequent months. After the animals receive the protocol, it is recommended the initial follow-up, with an interval of 3 months. According to the evolution, the prescriber will gradually increase the interventions interval. Between one prescription and another, the animals must have the confirmation on the interruption of the previous treatment for the beginning of the effect of the new protocol, and this procedure is quite simple and explained in the training program.

The restriction to this methodology is the usage in animals, or near them, of substances containing camphor, essence of eucalyptus or mint, because it is known that the direct contact or olfactory access to camphor and essence of eucalyptus are capable of interfering, with partial or total interruption of the healing processes. In a productive system in the field, the access to animals can be direct or indirect to those substances. The typical direct access would be the utilization of any topic medication with camphor or eucalyptus, ointment with camphor for wounds, or any topical anti-inflammatory, injectable or orally taken regarding these compounds. The indirect access would consist in a person that suffered a contusion, administered a topical product containing camphor for pain relief (e.g. pomades, gel, arnica gel with camphor) or for a wound and applied ointment and had access to animals. These medications generally cause a chilly sensation when in contact with the skin, and to avoid this negative interference, all you have to do is to ask someone not to use the medicine or not to have immediate access to animals to run an odor or chilly sensation test. The product can be removed with the use of soap and water and must be well rinsed.

In order to the veterinarian to qualify for the usage of the Self-organization Factors of the Biofield, a previous training is necessary to follow-up the evolution of the updates, in the recommended frequency of at least once a year, even if through the distance learning platform. The training can be obtained through the BioFAO Institute [16]. The medication is produced

in Rio de Janeiro according to standards of the Brazilian Homeopathic Pharmacopeia, 3rd edition, by Alquiotupã pharmacy [17] which is the institution registered in medicines regulator local authority (Agência Nacional de Vigilânica Sanitária—ANVISA) and shipped with due care and diligence to Brazil and abroad through a qualified prescriber.

4.3. Symptomatic treatment

The symptomatic treatment can be made with the choice of the medicines by the person in charge. Experienced people in the handling of ultra-diluted medicines may have access to information, which were learned under the professional guidance of specialized veterinarians during the development process of a productive system of family agriculture, organic, anthroposophic, among others. Another way to learn is cultural, with relatives, nowadays parents and grandparent's generation that made good use of the past, or even learned some guidance through books or with their own specialized doctors to treat themselves, their children and relatives in common and repetitive situations. There are courses for lay people to use ultra-diluted medications, and the safest way would remain in the symptomatic modality; however, there may be lack of some elements for a proper evaluation and complete exploration of the potential offered by the tools. The symptomatic use would be, for example, treatment for sneezes, diarrhea or inflammations, but that could prevent the animal of having access to a deserved care, even if conventional. The experienced person in charge, in these situations, must be aware and know how to recognize the threshold to take actions between attempting a treatment of initial symptoms with resolution and the actuation of a professional to assist his herd, either for treatments with ultra-diluted medications or not.

4.4. Multiple medicines prescribed by a professional

The complexist method means the use of more than one drug in a certain period of time for an animal, individually, or in a group of animals. The qualified specialized professional collects the detailed information and performs the procedure of repertorization before the prescription. It is the most widely used system in veterinary medicine with ultra-diluted medicines in the world, especially due to its higher latitude of action with a faster expectation of resolution by people in charge due to the current demands. This method can even use the repertorization procedure, which consists of a specific search with more details in the next topic. A farm may have a small medicine stock guided by the specialized veterinarian for usage in cases in which it is necessary an immediate and specific use. Situations like, for example, a deliver with difficulties or an acute inflammation of the mammary glands may get unassisted if identified in the evening, out of the working hours, in distant places of the places where the medicine is administered or places that depend on shipping by remote delivery.

Besides that, in a certain dairy herd in which there is relatively low turnover of animals and greater identification of individual behavior during the milking is very suitable for the determination of the most proper medicines for each animal, which can be used according to the complexist methodology together with the general for the herd, for that phase of the herd with the requested organization and handling. The most interesting phases of caprine and ovine may be: Dehorning, if necessary in caprine, separation of the mothers and weaning of

caprine and ovine, mounting seasons, shearing, start or ending of the rainy season, slaughter, that is, alterations in the productive phase or general function.

This method, combined with the handling alterations has the potential to widely substitute the use of conventional medicines, allows a great use for collective occurrences, like common diseases in small ruminants, like mycoplasmosis, caprine arthritis encephalitis, lymphadenitis, behavior changes, among others. This is the most well-known methodology among specialized veterinarians and which has a worldwide availability, and it can be used in agroecological, organic and complimentary to anthroposophic systems, among many others. It can make use of more well-known medicines, less well-known medicines or even produce specific medicines for the herd from material collected from the own animals (biotherapeutic). In synergy with the usual or even conventional ultra-diluted medicines, the biotherapeutic can control and initially stabilize a disease process of the herd and can subsequently evolve and change according to the evolution and the safety provided to the animals. In case there is usage of conventional combined medication, the tools offered by the complexist method can slow the healing time for the animals, reduce the risk of recurrence of symptoms, increase tranquility and reduce stress normally present in the productive systems.

The purchase of the medication will depend on the offer and local legislation; there can be restrictions and more severe control for the application of medicines in animals that produce meat and dairy products. Several countries in Europe have access to medicines produced by Boiron Laboratory. In Brazil, there is a specific regulation for laboratories to manipulate homeopathic medicines for production livestock. However, since there is a tolerance that varies according to the region and city in the usage of manipulated medicines in drugstores for humans in big pharmacies for pet shops, it is common that this place is a reference site indicated by professional veterinarians that prescribe ultra-diluted medications.

4.5. Single medicine personally prescribed by a professional

The classic method is known by the use of only one ultra-diluted medicine per animal or for the entire herd in a certain period of time. The qualified specialized professional will collect detailed information and carry out the repertorization procedure before the prescription. This is the most traditional method of all methodologies with ultra-diluted medications, is detail oriented in the data collection for the execution of a repertorization procedure. The determination of the most adequate single medicine, also called background medication, among a great variety of medications, is imbued in the task of identifying the substance that will characterize another health-disease process of each animal or collectively of the herd as a living system. It is quite suitable for animals in expositions, competitions, animals that have a high zootechnical value and animals with narrower conviviality with human beings either in dairy production or as pets, and animals that participate in specific activities, like, for example, in goat yoga.

This methodology is considered a gold standard by many professionals of the classicist thinking, and the animals can make use of the same medication in several different potencies and frequencies according to the evolution of facts. The main objective is the determination of the

archetype that the animal or certain herd is actually facing, therefore it is used preventively or in a healing way, but the historic of the health-disease process is important to determine the strategies of a treatment by the single medicine method. Even if a little bit usual from the perspective of people in charge of animals, the specialists believe that it might develop an even better job if the animals, individually or in herd could be followed-up as soon as possible, since the gestational process of the next generation or even being young. The observation of the behavior patterns, even if the animals are not ill, provides data for the specialized veterinarian that will be considered in repertorization. The previous treatment of the animal, did preventively, may increase its metabolic resistance, minimize the risk of getting ill and negative effects in case of an outbreak, and even reduce the use of work force and additional medicines.

The establishment of a single drug by repertorization will need to interview someone that knows very well the animals, generally those who are in charge that directly handle them will describe differentiation details which are singular among the animals of a herd or from a herd to another. These people know the individuality and the behavior in details, besides the history of illness, if available. The repertorization is a procedure that in the old days was made manually by consulting a catalog of peculiar symptoms. These symptoms have a description in specific notation called repertorial language and may depend on the interpretation of a professional used to the described information. The inexperienced observer may not know the reality between the incidence of phenomena with animals, the repertorial language and its interpretation and mistakenly believe that a medicine is suitable.

Nowadays, there are several solutions in softwares that make faster the determination of a single drug, which have to be carefully evaluated by the attentive professional. The softwares may always bring the medicines with more cataloged symptoms in detriment of the others, preventing an eventual identification of a singularity. After the determination of some of the possibilities of more likely medicines, each one will be consulted in the description catalog entitled materia medica to confirm the previous results. This confirmation is qualitative, thus is subject to several opinions according to the experience or diversity of the collected reports in an opportunity to collect information. The medicines used in the unicist modality may be re-evaluated by the professional certainly much before judging by adjustments or mistakes; the process will have to evolve in order to guarantee that the animals have their needs met for its health and balance. The professional that follows-up this treatment will follow-up and give initial systematic feedbacks, when he or she will gradually increase the intervals between appointments with the evolution stabilization.

Due to pressured and demands of today's world, the people in charge have requested treatments with fast responses, without feedbacks, with a low offer of tolerance regarding handling alterations or extra jobs beyond the current ones. Short-sighted solutions and those in the short term may be a practical ideal for people in charge of the animals, but not necessarily for them. Chronicle and long processes, regardless of used therapy, will demand financial and work force investments given the necessary attention of the issue. The exclusively unicist methodology used in veterinary medicine may have less supporters nowadays regarding the complexist methodology, but the repertorization procedure with the identification of the background medicine is extremely useful and remains being applied in both modalities.

4.6. Cancer symptomatic support prescribed by a professional

Animals with a high zootechnic value or pets may demand treatment for an eventual incidence of neoplasia. All goats and sheep can have their neoplastic processes treated with ultra-dilution medicines; however the investment in this kind of treatment according to the activity regarding the animals may present a low cost-benefit due to investments. Besides that, the malignant neoplasia cases are considered incurable diseases and hard to be controlled, thus they will demand a different attention from the specialized veterinarian because it might require frequent follow-ups. Though, for animals with high zootechnical value, this reality can be feasible, because they are generally used for the collection of embryos, participation in expositions, storage of the genetic material *in vitro* of breeds and lineage at risk or little preserved, of high interest in terms of genetic material. Besides that, caprine and ovine taken as pets may have the desired treatment by personal interest due to emotional involvement factors, either in a family nucleus or in an institutional environment with specific interests. The cases of neoplasia must be followed-up by a qualified professional, and the ultra-diluted medications can increase the life quality, modulate the evolution of the tumoral processes and decrease the suffering of animals in many situations.

There are specific protocols committed with the treatment of neoplasia, however all of them must be supervised by an experienced professional in the field of neoplasia and ultra-diluted medications. Generally, there will be a reference persona round experienced in the use of those tools. Examples of used medications are some potencies of Iscador® (mistletoe or *Viscum album*) [18], treatment with the repertorization of neoplasia, biotherapeutic of neoplasia, self-organization factors of the Biofield in a differentiated way [19]. The protocols can be used in combination with conventional treatments. The treatment of an animal with neoplasia may need a multidisciplinary team, attention and full-time dedication, as well as the referred investments resulting from the necessary support.

5. Conclusions

The treatment of goats and sheep with ultra-diluted medicines is applicable and recommended for any activity regarding these species. People who consume and use animal products wish a reduction in the exposure to residues of any kind, and the treatments with ultra dilutions specially contemplate this demand. The breeders of goats and sheep that wish to improve and prevent diseases, with an increase in the life quality of the animals, regardless of the size of the herd, can use ultra-diluted medicines. The usage of ultra-diluted medicines for goats and sheeps may depend on a follow-up by a specialized professional (homeopathic veterinarian) according to the demand. Opinions and researches coordinated and guided by at least one experienced and qualified professional in the team in the use of ultra-diluted medicines may be considered with special regard. Promotional and research material done by people without a proper formation in this field must be considered with restrictions.

Conflict of interest

The author has no conflict of interest to declare.

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Heat Stress

Chapter 15

Characterisation of Goats' Response to Heat Stress: Tools to Improve Heat Tolerance

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Additional information is available at the end of the chapter

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Abstract

This chapter aims to review present knowledge about the effects of heat stress on goats, summarising what is known about its measurement, its impact on the performances of the animals, mainly milk traits, the physiological and genetic bases of the animals' response and the improvement of resilience through selection. A short historic review of the climatic indexes used to measure heat stress, with special emphasis on the temperature humidity indexes, and the main consequences on milk yield and composition are followed by a description of the results of experiments carried out to study the physiological and metabolic consequences of heat stress. The results of the quantitative analyses of the genetic bases of heat stress using norm of reaction models and of the application of omic techniques, particularly transcriptomic and genomic, to understand the complexity of the genetic background of animal's reaction to thermal stress, constitute the next points. The chapter ends treating the possible ways and difficulties of applying selection to increase resilience to heat stress.

Keywords: heat stress, physiological response, genetic analysis, selection for resilience

1. Introduction

The extraordinary results obtained through artificial selection in all domestic animals had some undesirable side effects, such as low reproductive efficiency, increased susceptibility to diseases and higher sensitivity to sudden environmental changes [1]. In particular, artificial selection to increase milk yield has been proved to reduce heat tolerance in dairy cattle [2, 3] and dairy sheep [4]. Heat stress (HS) has also been proved to be one of the main causes of economic losses

[5, 6]. Temperature increase and rainfall reduction are expected during the next decades in many areas of the world and, particularly, in the Mediterranean region, where the largest part of goats in Europe are raised [7]; therefore, knowledge of the physiological and genetic bases of the response to the consequences of climate change is needed to reduce its impact. The study of physiological indicators and the quantification of the genetic variation of the response to HS, as well as the genomic and other –omic analyses to find candidate genes involved in this response and the changes in gene expression so induced, allow for the identification of animals with a positive response to HS and the design of methods to select them.

Research works described in this chapter aimed to ascertain the effects of HS on milk traits and to study the physiological and genetic bases of these effects in order to allow for the inclusion of resilience to HS as a new goal in the selection programmes of three Spanish breeds of goats.

2. Climatic indexes and characterisation of the effect of heat stress on milk and reproduction traits in goats

In order to know the effects of HS on production, a measurement of the climate effect on the animal comfort is needed. The first index, specifically developed for animals, to measure the stress produced by some climatic conditions was the Iberian heat tolerance test (HTC) [8], which was used to assess the heat tolerance of cattle by measuring how much rectal temperature exceeds the normal value of 101.0°F (38.33°C). Heat tolerance was determined by the value of HTC, so the higher the HTC the more heat tolerant the animal is perceived to be. This index was followed by other indices such as the coefficient of adaptability [9], the biochemical index of heat tolerance [10], the discomfort index [11] or the milk production decline index [12].

Johnson et al. [13] showed for the first time the relation of temperature and relative humidity with comfort and dairy performance in cattle, which is the base of the combination of temperature and relative humidity in an index, called temperature humidity index (THI). Some adaptations of the previously described indexes had followed (e.g. Bianca [14] adapted the HTC index to express rectal temperature in °C), Kibler [15] being the first to use an index with the current THI philosophy for livestock. The index was later refined on a number of studies. Berry et al. [12], for example, incorporated dry and wet bulb temperatures to the index. Since then, the THI (in its various formulations) has been widely used to assess the response of animals to heat stress.

Heat balance is a complex phenomenon affected by numerous climatic factors (e.g. ambient temperature, relative humidity, wind speed, radiant heat and other factors such as altitude), animal factors (e.g. age, genotype, hair coat characteristics, degree of acclimatisation, health status, physical activity, level of performance, reproductive state, etc.) and management factors (e.g. housing, provision of shade, fans and others). However, in its usual formulation, the THI index only reflects the influence of the temperature and humidity to which the animal is subjected, without considering other important effects such as thermal radiation (solar and long-wave), wind speed or the duration of the exposure to these conditions. The reliability of using THI to predict animal responses to thermal stress has been examined in Refs. [3, 16–18], showing all of them some limitations of the index, the major one being that it does not account

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for solar load or wind speed [19], parameters of great impact on animal physiology, especially in grazing species. In addition, it does not take into account the breed, the genotype or other animal differences (e.g. age, level of production). Gaughan et al. [20] concluded that the THI may not adequately describe the effect of hot climatic conditions on livestock (and much less on the effects of cold conditions). Furthermore, with the exception of the case of cattle, most THIs have not been specifically designed for their own species (and much less for a specific breed exploited in certain geographic and climatic conditions). Thus, in the case of the goat species, the scarce number of studies carried out so far has used THI formulations developed for beef and dairy cattle, with the exception of the works carried out by our research group [21–24] in which a modification of the THI developed for sheep exploited under Mediterranean environmental conditions by Finocchiaro et al. [4] was used. Only very recently the feasibility and the validity of a heat stress score specifically developed for intensive dairy goat farms has been tested [25].

It is generally recognised that goats are more tolerant to HS than sheep, and both are superior to this respect than cows, due to the morphological and physiological differences between these species related to heat dissipation [26]. However, it is also well known that high temperatures and relative humidity values affect productivity of small ruminants [27, 28]. As opposed to the case of dairy cattle, few woks have dealt with the effects of HS on milk yield and composition in goats. In several studies lately carried out to quantify these effects on native breeds of goats, Murciano-Granadina and Payoya [21], Florida [22], Malagueña [29], raised mostly in the South of Spain, it has been observed that the animals are exposed to stressing climatic conditions, due to high temperatures, during 45–55% of the year (**Figure 1**), generating losses of 1.9 and 3.1% of annual fat plus protein yields in Murciano-Granadina and Payoya goats, respectively (**Figure 2**). Sano et al. [30] found milk yield losses of 3–13% in dairy Saanen goats exposed to moderate or severe HS for 4 days (THI, 81 or 89), respectively. Brown et al. [31] reported that

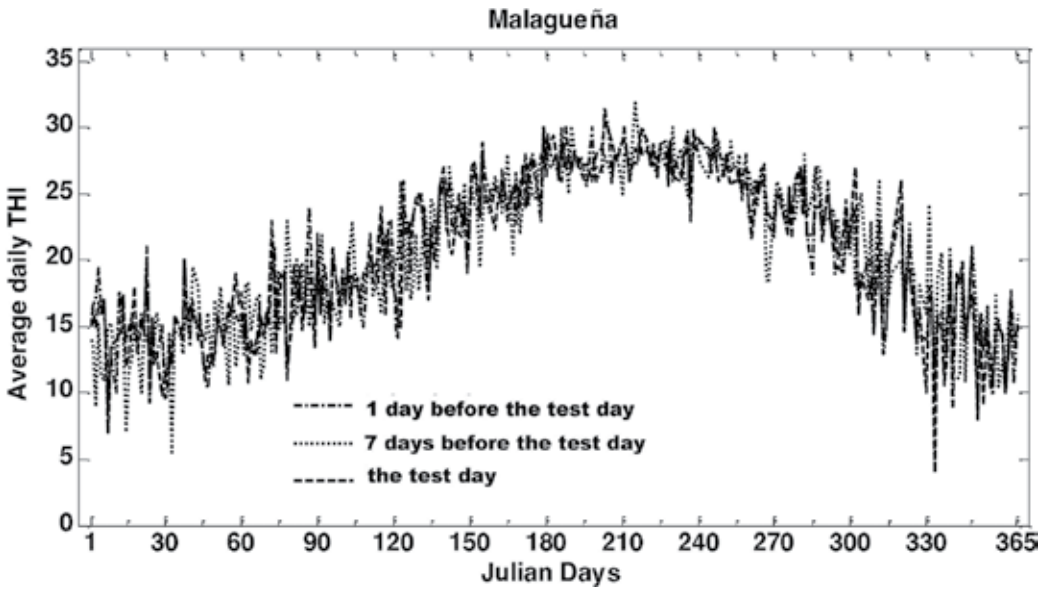

Figure 1. Evolution through the year of THI values in the South of Spain (from Ref. [22]).

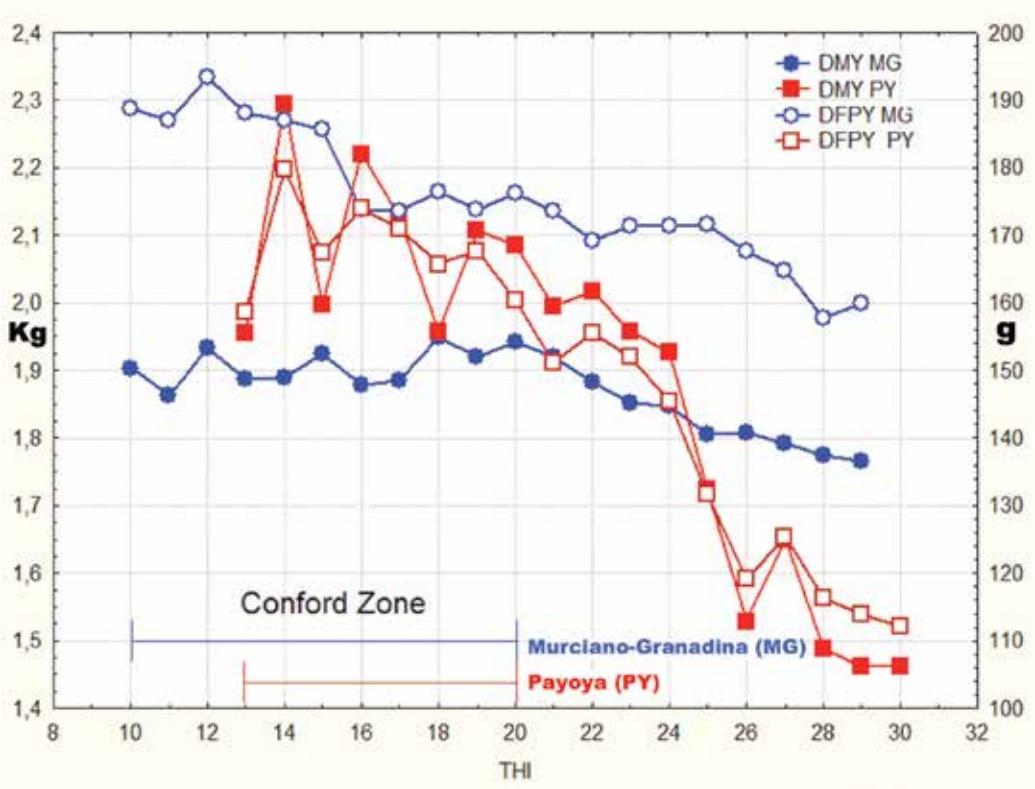

Figure 2. Graph of daily kg of milk yield (DMY) and g of fat plus protein yield (DFPY) as a function of THI for Murciano-Granadina (MG) and Payoya (PY) goats (from Ref. [29]).

the exposure of dairy goats to moderate HS conditions (THI = 79) decreased milk yield in Alpine but not in Nubian goats. Differences in the genetic potential for adaptive traits and also for production might explain these differences.

3. Physiological and metabolic response to heat stress under controlled conditions

Animals respond to HS reducing feed intake to decrease metabolic heat production and launching heat dissipation mechanisms like increased perspiration and respiratory rate. The combined effect of lower dry matter intake (DMI) and higher energy expenses to maintain body temperature may provoke a negative energy balance and a deficit of nutrients with negative effects on production and reproduction, as well as on the animal health status. In dairy cattle, only 40% of the reduction of milk yield has been proved to be due to a lower feed intake [32, 33]. Heat stress is accompanied by metabolic changes that are also responsible for the decrease in production. In dairy goats maintained in climatic chambers to generate a

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HS situation (THI = 77–85), results shown in Refs. [34, 35] indicate that although a substantial reduction in DMI (22–35%) coupled with an increased rectal temperature (+0.58 °C) and respiration rate (+48 breaths/min) were observed, reduction in milk yield was relatively low (3–10%) with reduced contents of fat, protein and lactose. As in cattle, the reduced intake was not accompanied by increasing levels of non-esterified fatty acids (NEFAs), which is typical in feed-restricted animals under thermal neutral conditions. In cattle, this seems to respond to a shift in the energy metabolism from using fat to using glucose as main fuel under HS [32, 36]. In dairy goats, the lack of fat mobilisation was not accompanied by decreased glucose levels and increased levels of insulin as it is in cattle [34, 35]. These authors launched several hypotheses to explain this different behaviour in goats, one of them being that the pancreas of HS goats is less sensitive, which could be a way to maintain normal glucose levels in blood. Overall, the effect of heat stress on goats seems milder than in highly producing dairy cattle and the metabolic consequences may be attenuated with respect to those in cattle.

The effect of HS on reproduction takes place through a reduction of oestrus duration and intensity [37, 38], malfunction of the axis hypothalamus-pituitary-ovary and low quality of the oocyte [39], anomalous spermatogenesis [40] and a bad embryo development [39, 41, 42]. Among the effects of HS on the health status is a higher risk of mastitis [43], but it is not clear that this is due to a direct action of the stress on the animal immune system or to higher rates of proliferation and survival of pathogens [26].

According to Silanikove [28], rectal temperature is the best physiological indicator of HS. Heritabilities between 0.12 and 0.22 were estimated for rectal temperature by Prayaga and Henshall [44] in Australian beef cattle and an estimate of 0.17 was obtained by Dikmen et al. [45] in dairy cattle. These heritabilities permit to expect a positive response to decrease rectal temperature under HS conditions, as it was proven by Burrow and Prayaga [46] in a selection experiment also carried out in Australian beef cattle. However, rectal temperature is not an easy trait to be routinely registered in a large population; therefore, most of the quantitative genetic analyses of the response to HS have used bioclimatic indexes reflecting the level of thermal comfort of the animal. One of the most used is the formerly described temperature humidity index (THI) combining dry bulb temperature and relative humidity, proposed by Kelly and Bond [47].

4. Quantitative genetic analyses of individual reaction norm

The current approach to the quantitative genetic analysis of the response of milk traits to HS uses frequently random regression models based on the concept of norm of reaction: the different phenotypic expressions of a gene in different environments [48]. The magnitude of the change in the value of a given trait from one environmental condition to another measures the plasticity of an individual for a given trait and it is also a measure of the genotype by environment interaction. According to their norm of reaction, animals can be classified as stable or robust for a certain trait if the value of that trait remains constant through the range of values of the environmental variable and unstable or plastic if the value of the trait changes.

These models include two types of quantitative variables: the test-day values of milk traits (yield, contents of fat, protein or any other milk component) registered periodically to each animal in each farm and the values of a certain climatic variable (most frequently THI) registered in meteorological stations located the closest the possible to the farm. Misztal [49] and Ravagnolo et al. [50] were the first to apply this methodology for the estimation of the genetic components of the response to HS in dairy cattle. The model proposed by Misztal presented the test-day milk yield as a function of the THI with an independent term or intercept, standing for general genetic component of the trait, and a random coefficient or slope, representing the specific response of daily milk yield to a unit increase in the THI, which can be considered a measure of the susceptibility to HS. This model has been later modified including an individual threshold value of the climatic index considered, below which the animal is in a comfort state with no effects of HS. This threshold is different for each animal [51, 52].

Quantitative genetic analyses of the response of milk traits to HS in small ruminants are scarce, as opposed to those in dairy cattle. The first study carried out in small ruminants presented the results of a study on dairy sheep in the Mediterranean region [4], showing similar results to those observed in dairy cattle. The first application to the study of the effects of HS on milk traits in goats was performed in the region of Andalusia, in South Spain, in a native dairy breed (Payoya) raised under semi-extensive conditions [53]. A modified version of Finocciaro's THI and the selection criterion of the breeding programme of the breed (protein plus fat yield) were used as climatic variable and as quantitative trait, respectively. Genetic variation for the response to the increasing values of THI was found, with some animals showing a stable genetic response through the range of values of THI and others showing a significant reduction of their breeding value for the trait under HS conditions.

Later studies went deeper into the analysis of the response of milk traits to HS in other native Spanish breeds: Murciano-Granadina, Malagueña, Florida and Payoya (**Table 1**). A real comfort zone under a given THI value cannot be observed, but a smooth increase of the level of production up to 20–30 units of THI and a negative slope afterwards as a consequence of HS was found. The estimated average loss of fat plus protein yield as an effect of HS ranged between 1.9 and 3.1% [21]. It is easy to understand the importance of the economic loss in a region in which the animals might be under HS condition an average of 140 days per year. Menéndez-Buxadera et al. [21, 29] described the change of the components of the genetic variation, the heritability (h^2) and the estimated breeding values (EBVs) throughout the scale of values of the THI.

	Murciano-Granadina	**Malagueña**	**Florida**	**Payoya**
Years	2000 – 2006	2006 – 2012	2006 – 2012	2002 – 2007
No. of milk records	63,640	160,067	129,450	81,625
No. of animals in pedigree	6037	14,089	12,268	9917
No. of herds	20	17	20	18
Average daily milk yield (kg)	2.06 ± 0.93	2.01 ± 0.96	2.30 ± 1.10	1.89 ± 0.83
Average daily fat + protein yield (g)	176.4 ± 80.48	191.60 ± 82.13	189.20 ± 83.2	161.6 ± 71.0

Table 1. General statistics of the data used in the heat stress studies of native Spanish breeds of goats (from Refs. [22, 29]).

The general form used in these works for the estimation of the variance components is a random regression model including the combination of herd and date of recording of the trait, the combination of age and parity number, the number of kids born, and a fixed function of the covariables of the THI trajectory (modelled with a Legendre polynomial) as fixed effects, and the additive genetic function of the animal with milk records and their parents without data, the permanent environmental function of the animal with milk records through the THI trajectory (modelled with Legendre polynomials) and the residual (with homogeneous variance) as random effects. With the variance components estimated in these analyses, it was possible to compute the heritability (h_i^2), genetic correlations ($r_{a_{ij}}$) for each trait and for all points (i and j) of the trajectory of the THI values and the EBV for any animal with milk records or without milk records if it is in the pedigree.

The main results obtained are similar in the four studied breeds and they can be summarised as follows:

1. In general, the genetic variances for the intercepts (defining the overall genetic value for the trait that does not change with the heat load) of the functions of responses of milk traits to THI were much higher than those of their slopes (specific HS tolerance). The genetic covariances between intercepts and slopes were negative, indicating that high-yielding animals are less tolerant to HS. The covariances between the intercepts and the slopes for the permanent environmental effects were also negative.
2. Heritability estimates varied through the scale of values of THI, as can be seen in **Figure 3**. The pattern is very similar in all four breeds; therefore, only the estimates of h^2 in Murciano-Granadina and Payoya goats are presented in **Table 2**.
3. Genetic correlations between adjacent points in the THI scale are high (over 0.90), whereas these values are low between distant THI points, reaching values below 0.80 which is the threshold value proposed by Robertson [54] as indicative of a significant genotype by environment interaction (G×E). This implies that the genetic potential for production under hot and cold conditions is ruled by at least partially different genetic backgrounds. The ratio between the variances of the slopes and the intercepts indicates the magnitude of this interaction. **Figure 4** presents the values of genetic correlations (r_g) throughout the THI trajectory in Malagueña and Payoya breeds. Permanent environmental correlations (r_p) between pairs of points in the THI scale showed a similar pattern to those of r_g. The pattern of these correlations is an indicator of the persistence of the expression of the performance of the animal through the THI conditions. Differences between the four studied breeds were observed to this respect, PY being the breed with a better ability to adapt to stress conditions. This breed is raised under a semi-extensive production system and its average milk yield is lower than that of the other breeds which are raised under more intensive conditions.
4. The EBVs are not constant throughout the range of values of THI, as can be seen in **Figure 5**, which represents the evolution through the scale of values of THI of EBVs of the 100 animals of Murciano-Granadina breed with the highest EBVs at THI = 31 (a value corresponding to HS conditions) and the 100 animals with the lowest EBVs at the same THI. Three types of responses to THI were observed: non-tolerant animals, with their EBV decreasing as THI increases; robust, with EBV independent of THI and tolerant, with EBV increasing as THI increases.

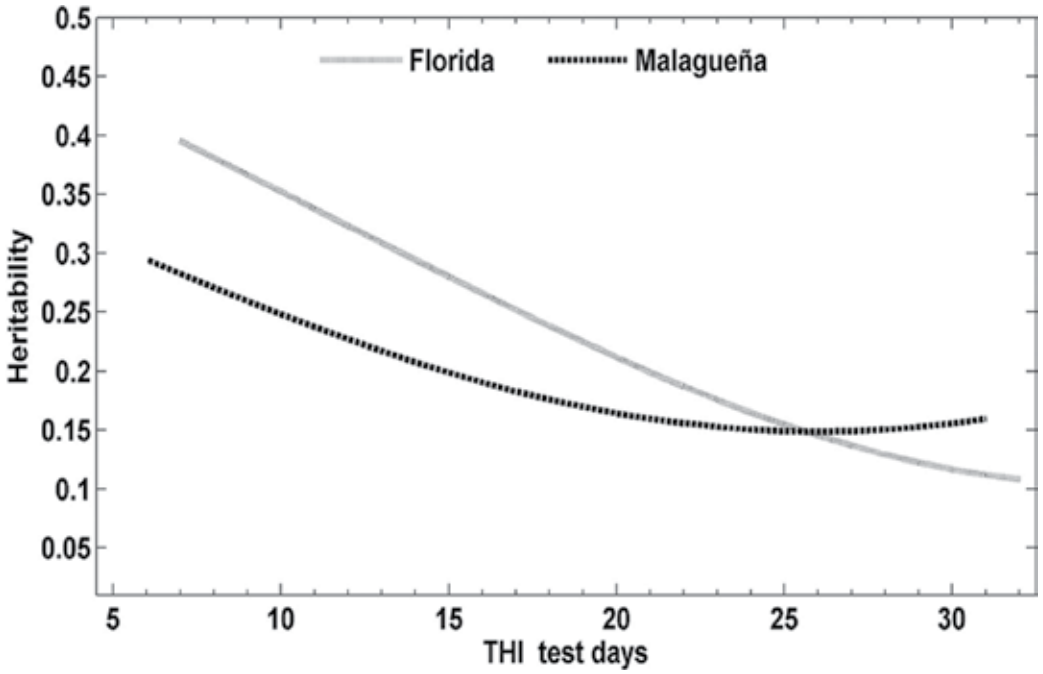

Figure 3. Change of the estimates of h^2 along the scale of values of THI in Malagueña and Payoya breeds (from Ref. [22]).

As a consequence of the differences in the estimates obtained for the variance components and the EBVs along the scale of THI values, any of the studied milk traits cannot be treated as the same trait throughout this scale. This is particularly important in respect to the estimation of breeding values, because the conventional methods of estimation are ignoring these differences and estimating these values at a fixed THI value; therefore, not tolerant animals might be selected. This genetic variability for the response to climatic conditions can be used to select the most adequate animals (tolerant or robust) to cope with future climate changes.

	h^2 comfort	r_g between zones	h^2 stress
Murciano-Granadina			
DMY	0.27–0.30	0.69–0.85	0.22–0.28
DFPY	0.21–0.23	0.71–0.85	0.20–0.22
Payoya			
DMY	0.21–0.22	0.71–0.90	0.22–0.24
DFPY	0.19–0.20	0.72–0.91	0.20–0.21

Table 2. Range of heritability (h^2) values of daily milk yield (DMY) and daily fat plus protein yield (DFPY) in the comfort and stress thermic zones and genetic correlation (r_g) between zones in Murciano-Granadina and Payoya goats (from Ref. [29]).

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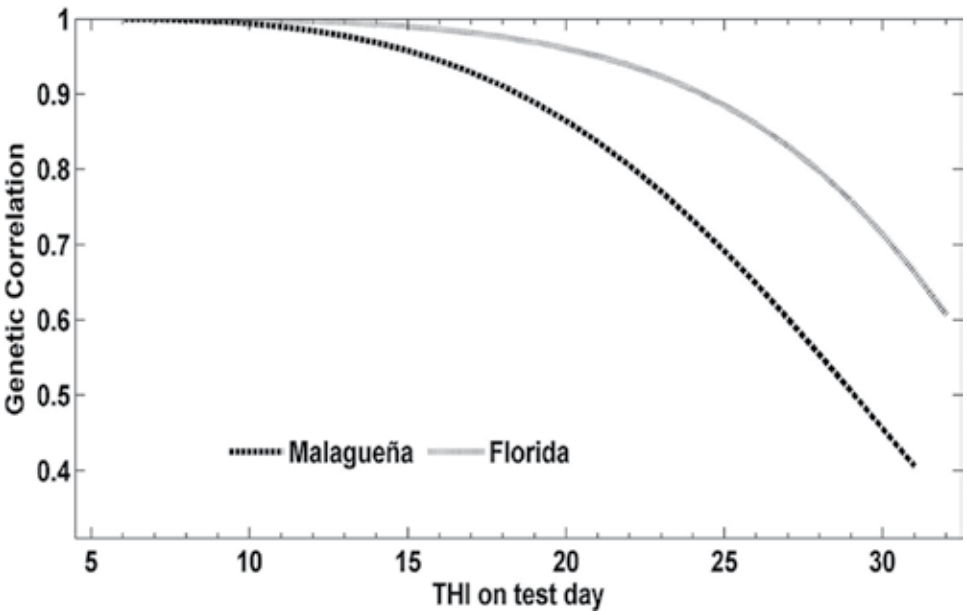

Figure 4. Genetic correlations of daily fat plus protein yields at THI = 7 and the same trait at all other values of THI in Florida and Malagueña goats (from Ref. [22]).

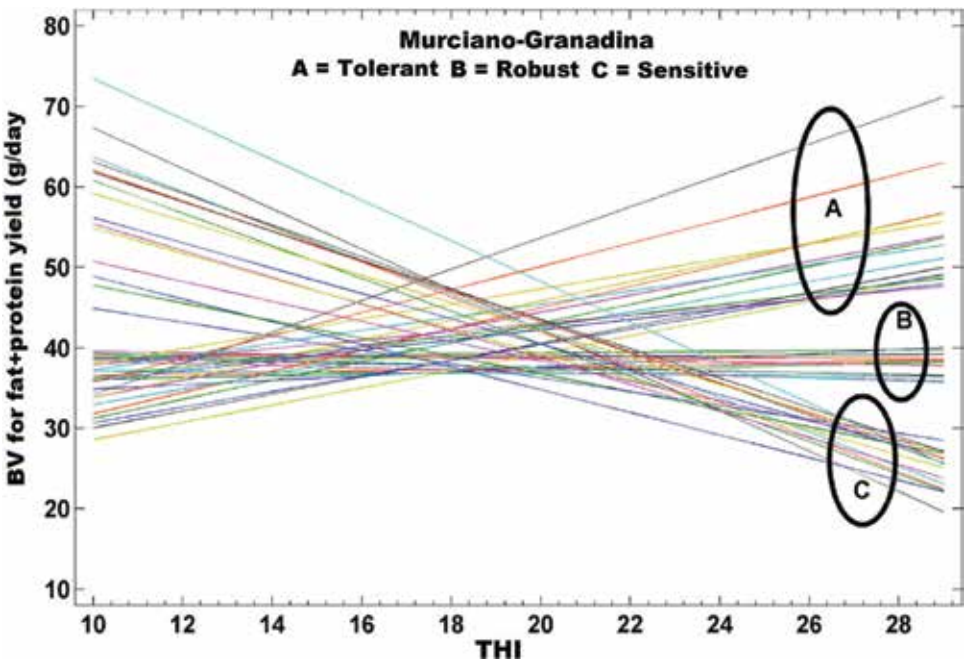

Figure 5. Evolution through the scale of THI values of the EBVs of the 100 animals of Murciano-Granadina breed with the highest EBV at THI = 30 and the 100 animals with the lowest EBV at the same THI (from Ref. [55]).

5. –Omic techniques to analyse the response to heat stress

Quantitative genetic analyses presented earlier have been used for the assessment of the animals' response to HS through its effect on milk production and fitness traits [21]. As a result, we can conclude that the genetic component of response to climatic constraints is not negligible and, therefore, to include traits related to thermotolerance of the animals in breeding programmes may help to face the challenge of climate change on goat production.

The development of -omic technologies has provided some powerful techniques to characterise the response of the animal to HS, either by evaluating (co)-expression patterns of genes in specific tissues [34, 56] or as a tool to understand the complexity of the genetic background of animal's reaction to thermal stress.

Several studies to dilucidate the genetic mechanisms underlying the response to heat stress have been performed using metabolic cages analysing patterns of gene expression occurring in blood and milk with either microarrays or RNA-seq. In blood, Hamzaoui et al. [57] using Affymetrix GeneChip Bovine in Murciano-Granadina goats in late lactation identified 39 and 74 genes whose expression was up- and down-regulated, respectively, by HS ($P < 0.05$). These genes were mainly related to biological processes and, to a lower extent, to molecular functions and cellular components. Moreover, ingenuity pathway analysis detected important pathways related to cell proliferation and death, free radical scavenging, inflammatory response, lipid metabolism and glycolysis/gluconeogenesis. Transcription regulators affected by HS were SATB1 (global chromatin organiser) and PPARD (which might be related to insulin resistance). The HS elicited changes in gene expression related to transcriptional regulation and metabolic processes. On the other hand, gene expression in milk cells has also been studied using RNA-seq by Salama et al. [35]. These authors have showed how decreases in protein and fat in milk composition are accompanied by downregulation in the gene expression of casein, fat and lactose synthesis and upregulation in the expression of genes related to milk cathepsins. This is an evidence of how findings in quantitative studies are the phenotypic expression on the underlying genetic mechanisms.

In parallel to transcriptomic analyses, a number of genome-wide association studies (GWAS) have been run mostly in cattle with the objective of identifying genomic regions associated with the signs of response to HS, based on production traits [58, 59], or physiological signs such as respiration rate or rectal temperatures [60]. Ideally, joining transcriptomic and genomic information together will help to find causal mutations (eQTLs) useful to address an effective selection favouring thermal tolerance in livestock.

A comprehensive review of the main challenges of performing GWAS analysis for HS response in dairy ruminants can be found in Carabaño et al. [61]. These authors highlighted the difficulty of dealing with milk recording data to find a phenotype measuring heat-stress response independent from milk production level in dairy production (cattle, sheep and goats). Separating production level from tolerance to thermal stress is very complex in dairy cattle because both components show a high correlation but not as much in sheep and goats [61]. Principal components analysis can be used to find variables related with heat tolerance independently from production level [59, 62].

A number of studies conducted to detect genomic regions and genes associated with heat tolerance via GWAS, mainly in cattle [58–60], have pointed at genes coding for fibroblast growth factors (FGFs), dehydrogenase-reductase member 3 (DHRS3), involved in the embryonic development in humans, and junctophilin 1 (JPH1), whose expression has been found to be upregulated in the hypothalamus of chickens subjected to HS, as candidate to be associated with HS. Carabaño et al. [61] using a GWAS approach have also validated some of the gene families already found in the literature in relation to HS, such as heat shock proteins (HSP, DNAJ), heat shock protein factors (HSF), mechanisms of immunoresponse (IL and CD) or NADH dehydrogenase (NDUF) families, FMO and FDF coding for growth factor relevant in the remodelling of the mammary gland after lactation. These findings corroborate the complexity of HS effect, involving apoptotic, immunological and metabolic responses.

Less attention has been paid on this topic in goats, as evidenced by the lower number of studies found in the literature. The extensive nature of its production system and the lower production level compared to dairy cattle have led to the belief that HS effects would be lower in this species. Genes found to be associated with response to HS in goats include HSP genes, genes associated with production traits, regulating respiration rate or playing roles in heat generation among others. Thus, Zidi et al. [23], conducting a GWAS analysis in a Spanish local goat, identified an HSP gene, the kappa casein gene CSN3 and some genes encoding enzymes such as malic enzyme (ME1) or acetyl-coenzyme A carboxylase alpha (ACACA). Though candidate genes were coherent with what is expected, the approach followed for this analysis somehow favoured the presence of false-positive signals because the slope used as a pseudo-phenotype in the GWAS analysis was estimated based on EBVs obtained from a reaction norm model without de-regressing them [63]. In another study on Egyptian desert sheep and goats, Elbeltagy et al. [64] found the GRID2 neurotransmitter receptor as genes associated with HS, affecting neuronal apoptosis and PDLIM5 (ontogenesis), or the SLC27, NR2F6 and DRD2 that have been found to be associated with heat generation and detection of temperature stimulus or homeostatic processes. Finally, Carabaño et al. [61] found a relevant signal for fat and protein yields response to heat common to the three dairy species (cattle, sheep and goats) pointing out to a region in Chromosome 6 where a gene encoding a member of the potassium channel-interacting proteins (KCNIP4) that regulate processes of defence against hypoxia and associated to hyperactivity disorders in humans.

Future works on -Omics could contribute to develop powerful tools to select animals not reactive to thermal stress; however, finding phenotypes of thermal response is still a handicap. Genome analysis of HS response should take advantage of the new technologies recently implemented for measuring biomarkers and proxies of thermotolerance in animals.

6. Improving resilience to heat stress

The studies on the genetic variability of the response to HS in dairy cattle [50] opened the opportunity to reduce the unfavourable effects of that stress on milk yields through selection, which represents a relatively cheap way (once milk recording and weather data are

available) that can complement the ways of reducing temperature in farms through costly heat abatement systems. Formerly described results show that applying random regression methods to the data from milk recording, together with the climatic information from the meteorological stations close to the farms, goats can be genetically evaluated for their response to HS and robust or tolerant animals can be selected. Selection criteria can focus on increasing the tolerance threshold or the slope of decay of the considered trait, but the estimation of the genetic value for the threshold of tolerance of each animal in goats is not easy due to the scarce and noisy information available (only six- to eight-test day per lactation) [61]. However, in all studies, in both dairy cattle and goats, it has been found that most of the observed variability of the response is associated to the production level (the intercept coefficient of the response) and only a small fraction of the variation is associated to the slope coefficient. Furthermore, there is an antagonistic relation between the intercept and the slope. Therefore, selecting for a lower decay of yield (lower negative or positive slope) would lead to a negative effect on the level of production. Carabaño et al. [61, 62] have proposed using canonical variables resulting from the eigendecomposition of the additive genetic random regression coefficient (co)variance matrices derived from the norm of reaction models formerly described. The canonical variable explaining the largest proportion of the variation of genetic values of animals across the range of values of THI for milk yield is linked to the production level and only a small proportion of about 10% can be used to select animals tolerant to HS without compromising the level of production. On the contrary, in the case of milk components there is a canonical variable explaining a larger part (up to 25%) of the genetic variation for heat tolerance independent of the production level. This could be a good selection criterion to get heat-tolerant animals for the trait fat plus protein yield, which is the most important trait in goats' selection programmes. According to Carabaño et al. [61], genomic information may play an important role in identifying genomic variants present in animals showing high production levels and a low rate of decay due to HS. Other possible tools to be used for selecting heat-tolerant animals could be the use of single nucleotide polymorphism (SNP) markers, found through GWAS analyses, associated with physiological indicators of the response to HS like rectal temperature and respiration and sweating rate. Biomarkers determined in milk by means of mid-infrared technology, routinely used for milk composition analyses in milk-recording programmes, and other recent discoveries of genetic mechanisms involved in heat tolerance through transcriptomics and proteomics may also contribute to find selection tools to improve the response to HS without impairing production levels [61].

7. Conclusions

Traditionally, it has been considered that goats are better adapted to semiarid and hot climates than cattle and sheep; however, the results of the studies carried out in native Spanish breed formerly describe showed that goats also suffer some physiological effects derived from the exposure to high temperatures with a negative impact on yields.

A negative effect on milk traits of both high and low temperatures was observed in three Spanish native breeds of goats. The effect is higher in high-yielding animals.

Genetic variability for the response to heat stress was observed. Heritability of milk traits and genetic values of animals diminish when heath load increases.

The GWAS and transcriptomic analyses showed some candidate genes possibly associated to the response to heat stress, which evidence the complexity of such a response involving apoptotic, immunological and metabolic process.

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Section 9

Production Systems

Chapter 16

Goat System Productions: Advantages and Disadvantages to the Animal, Environment and Farmer

António Monteiro, José Manuel Costa and
Maria João Lima

Additional information is available at the end of the chapter

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Abstract

Goats have always been considered very useful animals. Goats success is related to its excellent adaptability to the difficult mountain conditions, extreme weather and low value feed acceptance, versatile habits and high production considering their size. These are some reasons because goats are among the first animals to be domesticated. In terms of evolution, goats could be separated by their dispersion area in three large groups: the European, the Asian, and the African. Global goat populations, mainly in Africa and in Asia, have increased for centuries but very strongly in the past decades, well above the world population growth. They are also used for forest grazing, an integrated and alternative production system, very useful to control weed growth reducing fire risk. Despite some exceptions, no large-scale effort to professionalize this industry has been made so far. There are consumers for goat dairy products and there is enough global production, but misses a professional network between both. Regarding goat meat, the world leadership also stays in Africa and Asia, namely in China, and there is a new phenomenon, the spreading of goat meat tradition through Europe due to migrants from Africa and other places with strong goat meat consumption.

Keywords: goats, production systems, milk, meat

1. Introduction

Goats were among the first farm animals to be domesticated. According to Ref. [1], goats have been linked to humans for at least 10,000 years. Due to their great adaptability to difficult environment conditions and to different diets, they have always been considered very useful animals for their good productivity and easy to handle and they do not compete with man for food and eat cheap feeds.

In the last 50 years, global goat populations have increased by about 240%, while other livestock species have maintained or decreased their populations. Currently, there are about 1 billion goats around the world. More than 90% are located in Asia and Africa and only 1.8% in Europe. While in the countries, where goat production is massive, high adaptability to the environment is one of the main breed characters, in the developed countries, most of the goats are of genetically selected breeds for high production [2].

In rural areas of developing countries, the contribution of goats is highly valued and has an important role in feeding the populations, an item that is often not adequately recognized when comparing goats with sheep and cattle. In fact, goats are extremely intelligent animals, very agile, and independent, with a high level of resistance to diseases, much better than other ruminant species.

Despite the fact that in recent decades there has been a great progress in research aimed at increasing the goat productivity, there is still a great work to be done, namely in the applicability of the different races to the different environmental realities of the underdeveloped and developing countries. In these areas, the current and potential importance of goat production combined with the use of breeds that have not yet been fully selected and exploited will certainly be a reality in the near future. At both local and macroeconomic levels, goats will certainly be an animal production that will enhance the livelihoods and sustainable development of the world's poorest populations [3].

2. Material and methods

To prepare this theme, a documentary research was carried out to analyze world goat products and its production systems. The main sources of information were scientific papers, books, and statistical data from FAO.

This chapter covers the broader framework for goat's production systems, milk and meat production, and major advantages and disadvantages to the animal, environment, and farmer.

3. The origin

The origin of the current goat breeds is not clearly known. It is believed to have been originated from wild goats found in Asia Minor. The origin of the domestic goat is attributed to the wild species of the Quaternary: *Capra aegagrus* (**Figure 1**), *Capra falconeri* (**Figure 2**), and *Capra prisca*, being disseminated by all the continents and all originating from different regions of Asia [4, 5]. The main morphological elements of differentiation were related to the insertion, section, and configuration of the horns. The elements of comparative morphology and the interspecific crossbreeding experiments suggest that the bezoar (*Capra hircus aegagrus*) from Southwest Asia is probably its most significant ancestor. Scimitar horns are common and probably due to the influence of this species. It is believed that another species already extinct,

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Figure 1. *Capra aegagrus* [6].

Figure 2. *Capra falconeri* [7].

like the *C. falconeri*, would have given rise to the majority of the breeds of India and Central Asia, giving them characteristics such as the long and coarse coat and the predominance of the black color over the others [4].

According to the majority of the authors, the enormous variety of breeds (although much smaller than that of the ovine species) is grouped by their area of dispersion in three large groups or trunks: the European, *Ovis capra europaea*; the Asian, *Ovis capra asian*; and the African, *Ovis capra africana*. The European trunk belonged to *C. aegagrus*, the Asian trunk to *C. falconeri* and *C. prisca*, and the African trunk to *Capra nubiana*, another ancestral species. Some authors consider *C. aegagrus*, the only ascendant of domestic goats or the main progenitor. This one has long horns and is still found in the mountains of southwestern Asia [5].

The identification of the geographic origins and ancestral forms of the current goats has been subjective, since the scarcity of data requires extensive speculative exercises of considerable controversy. Theories that have broader consensus suggest that the origin of goats lies in a set of primitive goat populations that have evolved in the arid and mountainous regions of Central Asia and Southeast Asia. Unlike the sheep, goat migrations allowed their spread to the Southern Europe and to the Northern Africa without having populated the American continent [8].

Goats do not have as many breeds as sheep, and unlike them, they easily adapt to the harsh environments if they have the opportunity. Like the sheep, goats were probably among the first animals to be domesticated by man. These animals are also considered to be one of the first helpers of man, for in addition to providing manure, skins, hair for cloths and shelters, he gave to the primitive man meat and milk for food [9].

The goat must have been domesticated at the same time as the sheep in the East during the Neolithic period about 7000 BC, and traces of this species are found in the lacustrine cities of Western Europe, as well as in archaeological evidence and in excavations of the neolithic places.

4. Goat production in the world

Figure 3 shows the global distribution of goat production between 1994 and 2014 [10]. In the last two decades, we can see that the geographic areas with the largest goat production are located in the global African continent, in a range from Southeastern Europe (Greece and Turkey) to South Asia (India and China), passing through the Arabian Peninsula and, in the American Continent, where the highlight goes to Mexico, Brazil, and Argentina.

The production of small ruminants is largely rooted in some world regions, for both historical and religious reasons. This is the case in all Muslim countries where pork is not a food option, as also in the case in India where beef is also out of the food tradition, in both cases due to religion.

Soil and climatic matters greatly condition the animal species produced, benefiting goat production in poorer areas in terms of forage capacity and enhancing this species in areas with steep slopes and mountainous rocky areas. Sometimes goat production is the only possible, due to the excellent adaptability of these animals to the difficult mountain conditions. On the

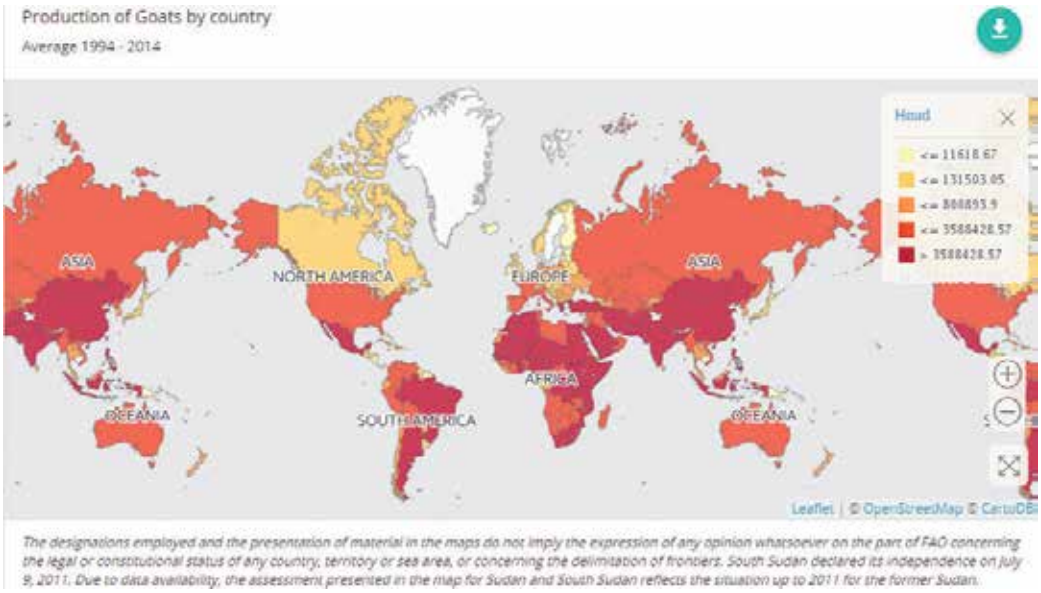

Figure 3. Global distribution of goats by country in number of heads [11].

other hand, forest grazing is an excellent productive alternative, associating in an integrated way the forest and animal productions. It helps to control unwanted weed growth with a consequent strong reduction of fire risk and, on the other hand, is often a valuable complement to the farmer or forest producer income, while it also helps to fertilize forest soils with their waste. To confirm this evidence, we can also verify in **Figure 4** that goat production reaches high levels in the most important forest and mountain areas.

In **Figure 5**, we can see similar results to those in **Figure 4**, but here we can see the distribution by animal density, in number of heads per km^2. Here we find the highest values in Mexico, northeastern Brazil, sub-Saharan tropical strip, India, and Eastern China.

Figure 5 shows that, only in the referred 20 years, the world goat population (from which there is a register) go from slightly over 600 million head in 1994 to over 1 billion in 2014, 67% rise in just two decades, much higher than the world population growth in the same period, which was about 28.5% [12].

The Asian continent clearly leads the world goat production between 1994 and 2014, a percentage well above the sum of the non-Asian world goat production (**Figure 6**). Then, we can find the African continent with about a third of the total, and finally, with very low percentage values, we have America, Europe, and Oceania, in this order, which make up, in total, about 7% of global goat production.

These figures complement and confirm those reported in **Figure 4**, where sub-Saharan Africa, China, and India showed their worldwide leadership in the production of this species.

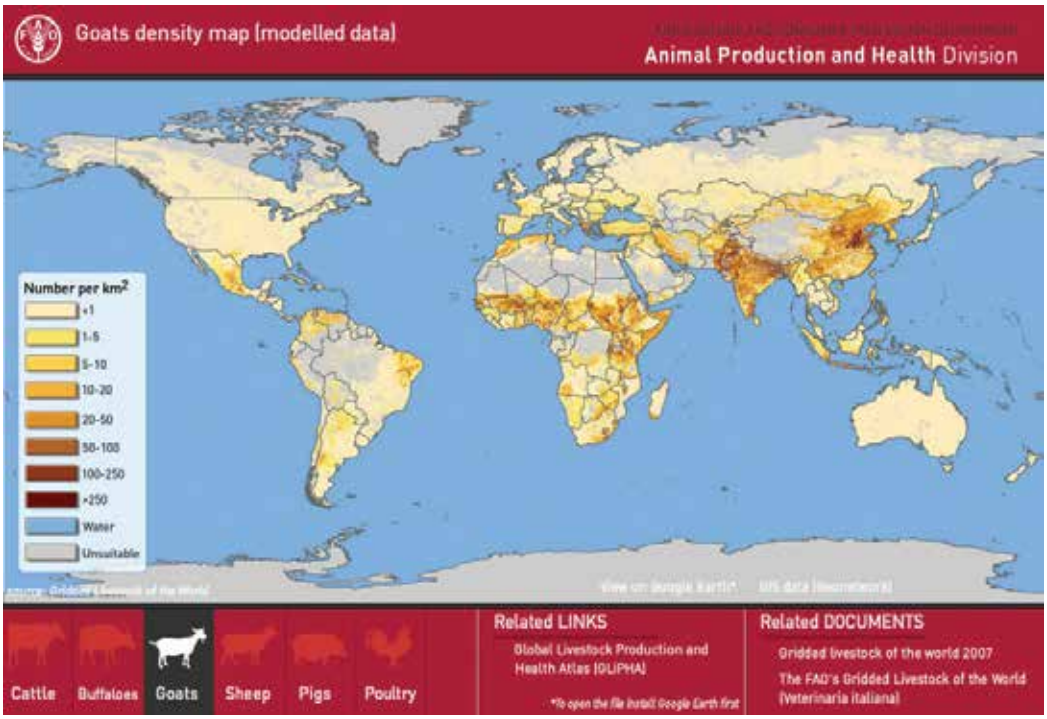

Figure 4. Worldwide distribution of caprine by country, in animal density [10].

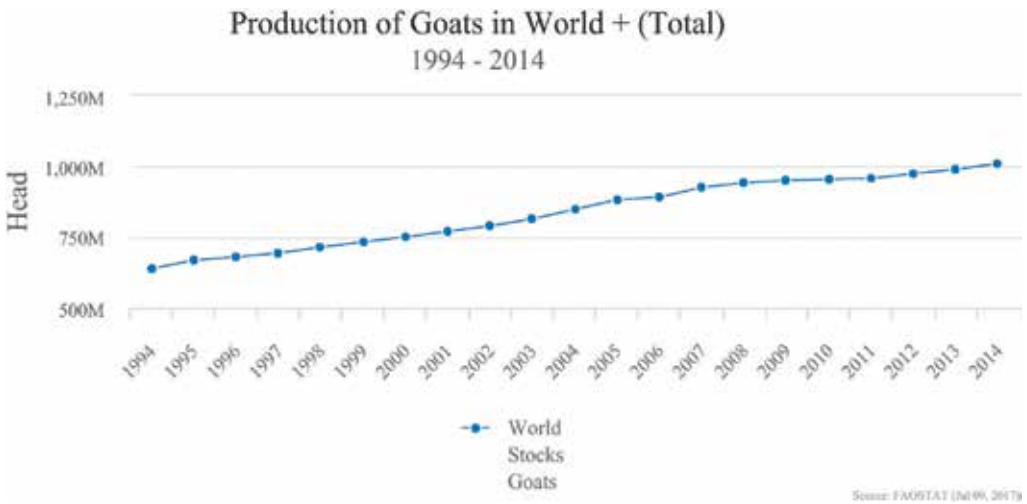

Figure 5. Evolution of world production of goats between 1994 and 2014 [10].

Goats are mainly grown to produce milk, meat, or fiber. Usually, the primary production is meat and milk, and there is little global information on fiber production.

The potential of goats for the sustainable supply of milk and meat for human consumption is unquestionable, and their contribution to improve the nutrition of rural populations is likely

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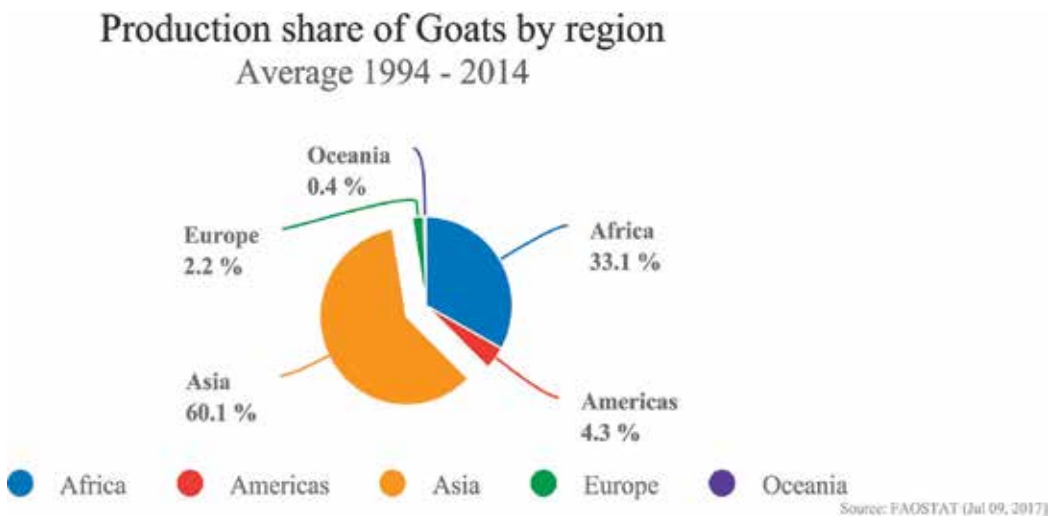

Figure 6. Evolution of world goat production between 1994 and 2014 [10].

to increase. At the same time, consumption of goat cheese should also increase in developed countries. This is attributed to the image of goat cheese as a product obtained under natural conditions when compared to cow's milk and its products, obtained from more industrialized farms.

Government programs to support goat rearing should focus on research and training on genetic improvement, farm management, infectious disease control, product collection and marketing markets [3].

4.1. Milk production

Unfortunately and unfairly, the dairy goat is still considered in some regions as the cow of the poor. In proportional terms to its size and feed intake, the milk yield of the goat is often remarkable. The goat has a reduced food intake, its feeding is cheap, it is a small animal and easy to keep, and each goat produces enough milk to feed an average family. Comparatively, and due to its size, the cow has very high maintenance costs and is more difficult to maintain at home.

Dairy goats produce about 15.2 million tonnes of milk, representing about 2% of the world's total milk produced by livestock species [13]. Developing countries, particularly in Asia and Africa, account for about 83% of the total value produced. In Europe, dairy goat farming is the main reason for goat production, where only 3% of the world goat population produces about 15% of the global goat milk, mostly for cheese production [14]. Spain, France, and Greece are the main European producers of goat's milk. In France, the development of research and development in the dairy goat sector has been intensified with programs organized for the selection, processing, and commercialization of goat's milk, mainly produced from the Saanen and Alpine breeds. In France, it is where the highest production figures per animal are achieved, whereas Iran has the lowest values.

China has the largest number of goats in the world, but they are bred mainly for meat production. Milk production by milk breed occupies the third place, behind France and Spain. According to China's official statistics, there are between 1.4 and 5.8 million dairy goats producing about 0.3 million tons of milk [13, 15]. The statistics on these goats can be questioned because they are not based on actual censuses, and knowledge of the number of dairy goats is even more difficult [15].

At the global level, interest in dairy goats has steadily increased, and in less than two decades, milk production has grown from around 10 million tons in 1990 to about 15.2 million tons in 2008. In developing countries, goat milk production has continued to grow partly due to a trend toward self-sufficiency of rural populations, where goat milk is the nutrition basis of millions of people. In these regions, milk is largely consumed raw but can also be processed into a wide variety of products for better preservation and commercialization. We can say that the global marketing of goat's milk is still at a very early stage, and so far, there has been no large-scale effort to professionalize this industry, exception for some developed countries. According to Refs. [15, 16], the marketing rate of goat's milk at the global level does not exceed 5%.

There are still many obstacles to achieve the professional level of production and sale of goat's milk. Access to productive data to enable the development of breeding programs is very scarce, and on the other hand, reproductive and productive seasonality, which is very marked in some breeds, prevents a constant supply of milk demanded by markets [17]. In sum, there are consumers for goat dairy products and there is enough production to meet this demand, but there is a lack of bridges between both sides in order to enhance this market in terms of an efficient marketing of the product toward profitable industry [3].

4.2. Meat production

Goat meat is widely consumed in developing countries. Of the approximately 280 million total tonnes of meat consumed per year, goat meat accounts for only 2% of this total, about 4.9 million tonnes [13]. Developing countries produced approximately 97% of this amount, reflecting the great importance of goat meat to feed these populations. China leads the world production of goat meat, representing 38% of the total. As we could see in milk production, also in goat meat production the Asian and African continents are leading. As in milk production, the increase was also notable in the same 18 years of comparative production and raised from the 2.65 million tonnes of goat meat produced in 1990 to 4.93 million in 2008. Most of this meat is not commercialized but is produced and consumed locally [3].

The goat meat business is not already become profitable on an industrial basis. It is not enough to know the proper management techniques, but nowadays it is also essential to control the market and the economic limiting factors for farming success, as well as the financial management and marketing techniques. In addition, there are an increasing number of challenges associated with meat production, including knowledge of regional preferences and consumer education for consumption. In the production and processing sector, producers have to undergo training programs, and slaughter and processing facilities have to be modernized.

Finally, there has to be made a strong commitment to organized programs of genetic improvement, market analysis, and marketing channels [18].

The increase in goat meat consumption has been occurring in some parts of the world due to the migrations of people who prefer goat meat to the developed countries. This recent fact has increased demand for goat meat in these areas [19].

5. Characteristics of goat production systems

Goat production systems, like other production systems, are not just a combination of crops and animals with the aim of achieving short-term results. They represent a set of interacting elements, managed by the farmer, according to their objectives. The farmer will define the objectives influenced by the social environment in which he is inserted, his degree of technical knowledge, and the available production factors [20, 21].

Several authors consider that the general and strategic management approaches are applicable to agricultural enterprises and those who apply them are more successful [22, 23]. The application of strategic management concepts helps to design the way the farmer will achieve the objectives, and it is convenient to know the strengths and weaknesses, the opportunities and the threats [24, 25].

A caprine production system has production subsystems, whose performance influences the overall results, for example, the forage system; if there are nutrient deficiencies in the soil, its production is lower, and then, the animals may not have adequate feed to their needs.

The increase in the meat and milk ruminant production has been much greater than the increase in the pasture area, mainly because this increase was due to the increase of mixed or landless production systems (intensive systems) than to the pastoral or extensive systems [26].

Increasing livestock production to provide food to the growing human population has increased the potential to cause environmental problems, and the balance between the environment and livestock production is now a concern. It is important to avoid an increase of grazing land and arable land for crops production to feed the animals, with the consequent reduction of areas of natural vegetation, soil and air contamination. Overgrazing also leads to several bad consequences like high amounts of animal excretions and overuse of natural pastures with low productivity and with high risk of soil degradation, especially in the arid and semiarid regions of the tropics and subtopics [26–28].

The terms equilibrium, disequilibrium, and nonequilibrium are commonly used and can be understood by equilibrium when the animal population and forage resources are under stable climatic conditions. Otherwise, climatic variations disturb the system, causing disequilibrium, which differs from nonequilibrium, represented by modifications in vegetation due to changes in the proportion in the plant species or where the dynamics of the animal population

is disassociated with key factors that determine their survival, and their survival is maintained through feeding supplementation [29–31].

Goats are the best adapted ruminants to highly antinutrifibrous low-protein forages, often in conditions of poor availability of water [32, 33]. Goats are opportunistic feeders, the time they spend on grazing species depends generally on the relative frequency of encounters, but this relationship depends on species of vegetation and habitat visited [34].

Compared to other domestic ruminants, namely bovine and sheep, raised in regions of poor agricultural resources, goats' advantage is clear. These animals combine the advantage of being able to feed on a variety of low-quality fodder and shrubs, they also manage to walk long distances, with short breeding intervals with high reproductive rates, they provide high rates of investment return and, consequently, low investment risk. Goats also have high energetic efficiency in milk production, excellent utilization of marginal lands, a very strong flocking instinct and a docile behavior, which enables herding by children and elders [35, 36].

Farmers tend to have mixed herds of sheep and goats as a strategy to maximize the use of environmental resources [37]. Small ruminants in many traditional systems in the Mediterranean basin are the main source of red meat for human consumption [38], while in Northern Europe, in addition to meat, the milk, wool, and skin were also the main products [39]. However, with development for a market agriculture, meat has become the main product in the sheep sector, while in the goat sector milk has been, and still is, the main product while meat is secondary in most cases followed by skins and hair [40]. There are, however, local variations where a particular product can be valued such as sheep's milk for the production of traditional cheeses and the production of goats in the southern Mediterranean. These products are mainly consumed regionally, constituting market niches with low international visibility.

Often, small ruminants, especially goats, are extensively produced using the poorest land, shrubland, and forest areas where other species cannot survive [41]. This helps to fix the rural population, reduces the risk of depopulation of marginal or less-favored areas [42–44], and contributes to the maintenance of good agro-environmental practices and landscape preservation.

6. Models of goat production systems

6.1. Extensive goat production systems

Extensive systems are characterized by large areas to feed the animals, with a low animal density. It uses soils of poor agricultural ability, located in mountainous areas with large rainfall or in areas of low rainfall, sometimes with extreme temperatures. There is a use of natural resources, made by autochthones goat breeds that are perfectly adapted to the environment, very rustic, but with low productivity.

The goat production in this extensive system uses family labor, often as a second source of income for families. The goat milk is obtained for family consumption or for cheese to sale.

However, the sale of the kid goat is the main reason for this production. In the Southern Mediterranean, this product is very appreciated and valued, mainly in the Easter and Christmas seasons.

Usually, the number of animals produced is low and they are usually raised under climate adversities, where probably there are no shelters, no food supplementation and also often these animals have hygienic sanitary problems, which leads to poor economic results.

It turns out that the use of natural pastures can be done in two ways, a mobile grazing system and a sedentary grazing system. The first is characterized by annual or seasonal movements of the animals with the shepherd to new places in search for feed, and while sedentary grazing, the animals are driven freely to pastures near the farm, usually keeping the animals at night in the stable.

In the mountains, they can practice transhumance, which consists of seasonal movement of animals regularly between two or more areas of seasonal pasture, through established paths (called "canadas" in Portugal and Spain), conducted by shepherds in the summer to the mountain to take advantage of the still fresh pastures and return to the valley in early autumn. This modality is currently disappearing due to sanitary issues and also because of the greater easiness to feed the animals through the purchase of commercial feeds.

Although the extensive production is not very productive, it is of great importance regarding the maintenance of the rural landscape and with the aim of the biomass management that avoids the occurrence of forest fires, and where the goats are well adapted to take advantage of these feed resources.

The use of goats in extensive systems can be valued by the quality products. In Europe, many of them have protected designation of origin (PDO) and the protected geographical indication (PGI) which are certified to attest the traditions and specific product qualities, strongly linked to a certain region. The awareness of society about the damaging effects of intensive livestock systems has changed the methods and aims of researchers and even research centers, trying to focus on improving system sustainability instead of increasing productivity [45].

6.2. Intensive goat production systems

Intensification is often associated with a decrease in grazing dependence and an increase in the use of concentrated feeds, mainly cereals, to supplement natural feeds. At the same time, improved and balanced feeding practices together with improved breeds in ruminant systems enabled more efficient feed ratio conversion to meat and milk production rather than to maintenance of the animals [28].

The intensive system implies a high density or animal concentration per area unit, under reproductive and sanitary control, and the feeding process includes advanced technologies. Sometimes, some farms seasonally require higher feed and labor resources such as through the calving season and milk production, so the supplementing of animals with concentrated feed may be needed at this point, but the remaining year is mainly grazing.

In order to meet the feed requirements of animals in an intensive system, pastures must have high dry matter yields per hectare, good growth throughout the year, in both regions with regular rainfall or holdings with irrigation systems, without extremes of heat or cold temperatures.

It is important that the breeds used in the intensive system have a high fertility and growth rates than the adaptability to the environment, that is, the rusticity. The equilibrium with the environment can easily be compromised by the insertion of exotic breeds, as well as with exotic or genetically modified plant species. The intensive use of natural resources may lead to their depletion and increase the environment pollution, having serious social consequences.

Goats are well adapted to harsh environments but can also be used in intensive systems with permanent housing, as is happen with many farms for milk production. Here too, mainly because these farms are usually in regions with weak resources, they use less labor but with higher qualifications, with the danger of triggering a process with social negative consequences, namely with soil erosion, high risks of fire and depopulation. Also industrial production can replace the artisanal production, losing ancestral traditions and biodiversity.

Intensive goat farms have higher costs for the installation and maintenance of production than extensive farms (**Table 1**), but their choice should allow for a balance between environmental, economic, and social factors, since only then will it be sustainable.

Extensive system	**Intensive system**
Eco-agrarian conditions	
Dependent on the climate (environmental) factors	Independent of the climate factors
Scarce resources	Constant forage production
Intermittent feed cycles	Continuous feed cycles
Parasitic problems	Good sanitary condition
Use of marginal land and nonagricultural resources	Use of technology
Low animal density	High animal density
Animal biotypes	
Autochthonous breeds	Improved breeds
Low productions	High productions
Multi-production exploration	Animals with high energy efficiency
Great ability of adaptation	Increased sensitivity to diseases
Lower fecundity and fertility	Increased fecundity and fertility
Environment	
Natural environment	Artificial environment
Long productive cycles	Short productive cycles
Traditional system	Industrial system

Note: Adapted from Refs. [1, 4, 8].

Table 1. Main differences between intensive and extensive production systems.

7. Conclusion

Concerning difficult environmental conditions, goats are probably the most well-adapted farm animals, and due to its cheap management and good meat and milk production, goats have been considered one of the ancient animals to be domesticated all over the world.

In several regions, namely in Africa and Asia, goats are the most important source of meat and milk to feed very large populations with scarce income. But these animals also contribute to a sustainable farming, a very ecological way of living which enables to use their waist to fertilize crop fields, control and prevent fires by forest grazing.

We can also find some intensive goat production all over the world, with selected high production breeds, namely for milk and cheese production, and in a near future, we will certainly need to spread the know-how from goat production research, applied to the different environmental conditions and its regional well-adapted breeds, enabling higher productions with lower effort, in order to feed more population from the developing countries.

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Chapter 17

Goat Farming and Breeding in Jordan

Ja'far Mansur Khalaf Al-Khaza'leh

Additional information is available at the end of the chapter

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Abstract

Goats are multifunctional species and play an important role in the livelihoods and food security of farmers particularly in rural areas. Goats are well-adapted animals to the various ecological zones in the tropics and dry zones of developing countries such as Jordan. In the country, goat farming is a major component of the agricultural system. This chapter provides an overview of the present status and prospects of Jordan's goat production sector, changes in goat populations, and includes an evaluation of constraints and their impacts on goat production in the region. It discusses the general trends occurring in goat raising, diversity, and characterization of the prevailing production systems. The chapter also focuses on the major breeds of goats and the differences and unique characteristics of each goat breed. Additionally, this chapter covers a considerable contribution of goats in terms of meat, milk, and culture to the socioeconomics of householders and its role in poverty and hunger alleviation in Jordan. Moreover, this chapter also discusses basic goats' productive and reproductive performance. Management calendar for goat production in Jordan is reviewed. Finally, the chapter covers goat health, diseases, and approaches or management practices for prevention and control of goat diseases.

Keywords: goat production systems, goat production constraints, goat breeds, goat health, economic performance, reproductive performance

1. Introduction

In tropical and subtropical regions of the world, goats are kept over other livestock because of their adaptive capacity, tolerance, performance, and importance to the livelihoods of the farmers.

In Jordan, goat farming is a major part of agricultural systems as goats play a significant role in the economy, food security, and livelihoods of farmers in rural areas. Locally, goats are the second most abundant ruminant livestock species after sheep, with a total number of goats to be around 857,728 heads in the country in 2014 [1]. The goat breeds in Jordan include Shami (Damascus goat), Mountain Black goat, Dhaiwi (Bedouin goat), and Desert goat in addition to crossbred goats. Goat breeds are distributed across different regions. Therefore, goats are raised in different production systems that are classified into sedentary (semi intensive), transhumant (semi-extensive), and nomadic (extensive) systems. Mountain Black goat is the main goat breed with the largest population. The proportional contribution of goats to households varies from breed to breed and system to system. Jordan is an Arab kingdom in Middle East region (**Figure 1**) with a total area of approximately 89,342 km^2 (land 88,802 km^2, water 540 km^2) and has a population estimated at 9.5 million people. The proportion of rural and urban population is around 16.3% and 83.7%, respectively [2]. The climate in Jordan is characterized by long, dry, hot summer and short, wet, cold winter seasons. Yearly temperature ranges from about maximum 26°C to minimum 13°C in winter to more than 32°C in summer; the temperature increases between 3 and 7°C toward the south, with the exception of some southern highlands. Rainfall varies considerably with location, mainly due to the national topographic profile. Between 1985 and 2014, the average annual rainfall in whole country was about 226 mm, with the lowest amount in the eastern and southern desert and the highest amount in the northern highlands [3]. Jordan's climate is, therefore, classified as semiarid with approximately 7% of arable land. Jordan is susceptible to climate change impact. Increasing temperatures, coupled with changing precipitation patterns, are expected to decrease water availability.

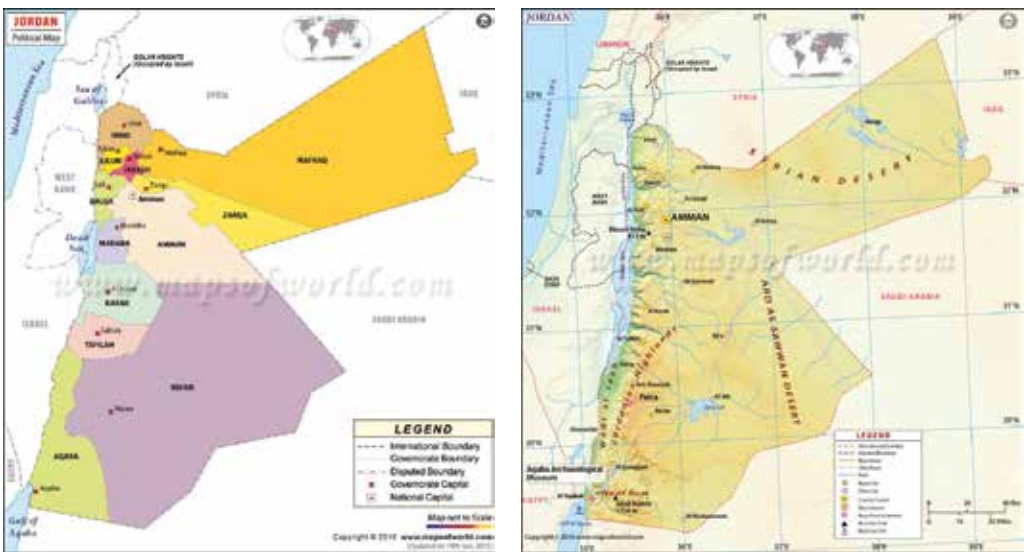

Figure 1. Map of Jordan and where Jordan is located in the world. Source: http://www.worldatlas.com/webimage/countrys/asia/jordan/jomaps.html.

2. Research methodology

The research methodology of chapter consists of a comprehensive, up-to-date compilation of available information on goat farming in Jordan. The methodological approach of the chapter involves the use of different elements, including (1) reviewing many articles and resource books in the field of goat science and searching the current topics in the literature related to goats in Jordan and (2) the experience of the author and consultation of other professors at different institutions in the region regarding goat production in Jordan.

3. An overview of the present goat farming status

Goats play an important role in the economy of the country. Goat farming in Jordan is the most important among all livestock activities in regions such as mostly desert plateau in east, highland area in west, and the Great Rift Valley separating the eastern region. Jordanian goat breeds are used to produce meat, milk, and milk products. Market demand for these different products varies. Goats contributed about 24.8 and 9.6% of red meat and milk yield of the total local production, respectively [4].

In fact, goat products are the basis of the livelihoods of farmers in some regions, particularly, in remote areas that have no other source of income. Goats are the only source of cash income, food, and savings for those households. Therefore, the maintenance of a viable goats sector is crucial to Jordan's economy. **Table 1** shows the estimated production value and quantity for goats in 2015. The goat meat production (newborn goats) in the country reached 610,243 head. The sheep and goat milk production (including dairy products) were 98,781 and 2496 (M.T), respectively.

The breeding organizations that should control the genetic improvement and management of the various breeds are still lacking. The current condition of the goat sector needs to be assessed and appropriate measures need to be implemented to ensure that it is developed sustainably. Goat meat production should be improved to meet the increasing demand for goat meat by human population.

3.1. Changes in the population of goats throughout the country

During the time period from 2000 to 2015, the goat population increased, reaching up to 860,220 animals in 2015. Most of these goats are owned by individual household. **Table 2** provides data

Type	Unit	Number or quantity	Value in JD
New born goats	Head	610,243	85,801,215
Sheep and goats milk	M.T	98,781	74,652,046
Dairy product	M.T	2496	17,576,606

Source: JD = Jordanian Dinar (1 JD ≈ 1.4 USD in 2015) [5].

Table 1. Livestock production value and quantity for goat holdings in 2015.

	Year			
Govern.	**2000**	**2005**	**2010**	**2015**
Amman	88,310	74,650	103,600	119,120
Balqa	35,150	54,350	82,310	91,220
Zarqa	46,260	43,830	41,790	50,720
Madaba	42,150	29,730	53,440	75,030
Irbid	29,180	47,190	48,940	75,280
Mafarq	40,710	69,560	80,240	97,000
Jarash	53,940	23,940	20,650	43,710
Ajloun	10,180	13,100	49,820	50,700
Karak	34,850	85,290	116,840	101,990
Tafilah	5590	18,560	36,210	30,450
Ma'an	30,380	28,800	77,550	87,140
Aqaba	55,760	27,140	40,340	37,850
Total	472,460	516,130	751,730	860,220

Source: [6].

Table 2. Number of goats by governorates during the period of time from 2000 to 2015.

on the changes that have occurred in Jordan's goat populations throughout the country from 2000 to 2015.

4. Goat breed characterization, regional distribution, and production system of goat breeds

Due to their behavioral, morphological, and physiological adaptations, indigenous goats are able to thrive under extreme temperatures and shortage of water [7]. Little information is available on the Jordanian goat breeds. Therefore, goat breeds have not been characterized well, and most of the information that is available has usually been gathered by the few researchers. Most of Jordan's native goat breeds are owned by farming households. They vary in morphological characteristics (**Figure 2**), production systems, regions, agro-ecological zones, household types, and herd size [8–10].

4.1. Black Bedouin goats

They are also known as Bedouin, Dihawi, Dwarf, and Hejaz Goat. They originate in Arabian Peninsula. Black Bedouin goats are small and have the lightest body weight. This breed of goat is known as "Dwarf breed" long time ago. The ears of Bedouin goats are medium in size and spotted white, most animals are black; however, brown individuals

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Figure 2. Morphology of Jordanian goat breeds. Source: Author and Dr. Tabbaa M. (2011, permission was obtained).

also occur. Black Bedouin goats are mainly kept in the southern part of the country (Petra and Wadi Rum). The Black Bedouin goats are adaptive and raised under nomadic (extensive) production systems under harsh environmental conditions of deserts. These breeds are mainly exploited for their meat.

4.2. Damascus goats

They are known by other names such as Damanscence or Shami Goat. They originated in adjacent Syria and are imported to Jordan because of their high productivity of milk and

twins [11]. They are used as dual purpose breeds for milk and meat. Damascus goat has a large body size. It has a Roman-shaped nose and is the most discriminating variable among different goat breeds of Jordan. Their ear type is pendent and has a high leg. They are reared around towns and countrysides of the northern part of Jordan and are kept under sedentary production system [10].

4.3. Mountain goats

They are also called by other names, namely Mountain Black goat or Balady (local). They are indigenous to Jordan and nearby countries. They are large dual-purpose breed and play an important role in meat and milk production in Jordan. They are distributed throughout all regions of the Jordan especially in the country's mountainous areas. They are kept mainly in semiextensive and sedentary systems. Because of their ability to survive and reproduce under the prevailing arid and harsh environmental conditions, this breed was used to develop highly productive crossbreeds especially with Damascus breed in Jordan.

4.4. Desert goats

They are indigenous to Syrian Badia desert. It is also known in Jordan as northern desert goat. They are light in size and mainly kept in northern-eastern Badia of Jordan and reared under extensive production system.

Figure 3 shows the relationship between goats breeds based on morphological-structural variables (nose shape, ear shape, presence or absence of horns and wattles, color, body

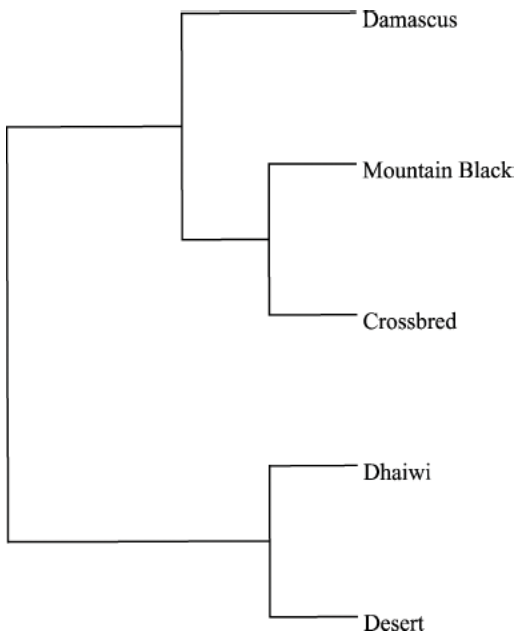

Figure 3. Relationship between goat breeds based on morphostructural variables [8].

weight, head length, and width, etc.). **Figure 3** shows two large clusters, first cluster includes Damascus breed as a large group and two subclusters of Mountain breed and crossbred goats, whereas the second cluster includes the Desert and Dhaiwi breeds.

Table 3 accounts for the distribution of goat breeds in the country. No information is available on the estimated population of Bedouin and Desert goat breeds of Jordan. However, Mountain Black and crosses accounted for the largest proportion of the current goat population.

	Breed	
Govern.	**Mountain Black**	**Crossbreed**
Amman	119,119	0
Balqa	91,223	0
Zarqa	50,718	0
Madaba	75,034	0
Irbid	75,139	139
Mafarq	96,997	0
Jarash	43,678	29
Ajloun	50,145	559
Karak	101,992	0
Tafilah	30,452	0
Ma'an	87,140	0
Aqaba	37,853	0
Total	859,490	727

Source: [12].

Table 3. Number of goats in the kingdom by governorates of different breed in 2015.

5. Goat performance

At birth, female kid's average weight is similar to male kid's average weight but male kids show higher weight gains during the suckling period. The body weight of males was significantly higher than those of females due to sexual dimorphism. The weight of adult goats has also been recorded by Al-Khaza'leh et al. [13] who reported that the mean body weight of adult does was about 45 kg with little variation ('5.4). The author also reported that the mean body weights of growing kids (of 1–11 months age) was 24.4 ' 5.7 ranging from 7.3 to 41.1 kg. Zaitoun et al. [14] reported a significant difference in mean body weight among the goat breeds, with the Damascus does being the heaviest, crossbred does having intermediate

weights and Mountain Black does having lowest weights. The differences in body weight of goat breeds were also recorded due to a region and production system effects.

Abu-Zanat et al. [9] reported that the lactation period usually lasts for 120 days from April to September with an average milk yield of about 40 and 67 kg per doe post suckling period for nomadic and transhumant systems, respectively, compared to about 81 kg per lactating female in the sedentary system. In the middle and northern Badia region of Jordan that is characterized by low and erratic annual rainfall, goats are mostly important for milk during the dry season [15, 9]. In general, goats have a lactation period of 300 days and produce about 100 kg of milk under extensive conditions [16, 17].

Damascus goats exhibit a high level of prolificacy [18]. In Jordan, the average prolificacy rate of goats is slightly more than 1 kid/doe bred per breeding season [19]. The information on the reproductive performance of goat breeds under transhumant and sedentary systems is displayed in **Table 4** [20].

	Production system						
	Transhumant (n = 76)			**Sedentary (n = 38)**			
Variables	***n***	**LSM**	**SE**	***n***	**LSM**	**SE**	***p*-Value**
Number of goats	76	91.7[a]	9.0	38	60.3[b]	12.8	0.047
Number of goats by breed							
Mountain Black	75	52.1[a]	5.3	34	32.1[b]	7.9	0.037
Dhaiwi	22	4.7	0.6	7	3.3	1.0	0.230
Crossbred	71	41.6	5.3	35	33.6	7.5	0.384
Goat flock performance*							
Fertility rate (%)	76	88.4	1.6	38	86.7	2.2	0.531
Flock mortality (%)	76	17.2	1.6	38	20.1	2.2	0.284
Productivity rate	76	1.03	0.03	38	1.00	0.1	0.588

Source: [20].

LSM: least squares mean; SE: standard error of the mean.

LSMs in the same row with different superscript letters differ significantly at $p < 0.05$.

*Fertility rate: number of does kidding at least once divided by the number of does in the flock during the year; productivity rate: number of kids born alive divided by the does kidding during the year; mortality rate: total deaths of goats in a 12-month period divided by the total number of goats in the flock at the beginning of the year.

Table 4. Performance traits of goat breeds by production system in Jordan.

As indicated in **Table 4**, the fertility and productivity rate per flock were similar between production systems. The barren rate was (12%) which is higher than that (6.6%) reported in the same study area by Aldomy et al. [21]. The proportion of barren does increases with age, regardless of the breed. **Table 5** shows the overall data on the fertility of Damascus does using different type of reproduction hormones [22].

	Treatment			
Variables	**CON (*n* = 8)**	**S (*n* = 15)**	**GP (*n* = 15)**	**GSP (*n* = 15)**
Kidding (rate %)	6^{b} (75%)	15^{a} (100%)	15^{a} (100%)	14^{a} (93%)
Kids/doe kidding (mean ′ SEM)	1.5^{b} ′ 2	1.5^{b} ′ 0.2	2.1^{a} ′ 0.2	2.0^{a} ′ 0.2
Kids/exposed doe (mean ′ SEM)	1.1 ′ 0.3	$1.5b^{c}$ ′ 0.2	2.1^{a} ′ 0.2	1.9^{ab} ′ 0.2
Litter birth weight (mean ′ SEM)	7.0^{ab} ′ 0.7	5.9^{b} ′ 0.4	7.5^{a} ′ 0.4	7.6^{a} ′ 0.4
Lambing date (mean ′ SEM)	147 ′ 3.6	152 ′ 2.3	153 ′ 2.3	149 ′ 2.4
Multiple births (no.)	$4/6^{ab}$ (67%)	$6/15^{b}$ (40%)	$13/15^{a}$ (87%)	$14/14^{a}$ (100%)

Treatment: No treatment (CON), progestagen sponges and equine chorionic gonadotropin (S), gonadotropin releasing hormone plus prostaglandin F2a (GP) or gonadotropin releasing hormone, and progestagen sponges and prostaglandin F2a (GSP).

^{a,b}Values within the same row with different superscripts differ ($p < 0.05$).

Table 5. Overall kidding data from mating during the induced and spontaneous cycles in Damascus does.

6. Goat flock management, husbandry, and constraints

Table 6 shows the details of the events and dates for goat production in Jordan.

Feed shortage, diseases, water shortage, high feed prices, rangeland shortage, poor veterinary service, poor breeding, and poor marketing are among the major constraints and challenges limiting goat production in Jordan. A study by Al-Khaza'leh et al. [23] showed that the most important problems confronting goat productivity in mountain zone of southern Jordan were feed shortage followed by disease, drinking water shortage and high feed prices while in the semidesert zone the top ranked constraint was high feed price followed by feed shortage, rangeland shortage, and water shortage. Al-Assaf [24] reported that inadequate feed and water supply, poor veterinarian services and weak management practices are the main challenges for goats in Jordan. Under the arid climatic conditions such as Jordan, it is obvious that the aforementioned constraints namely shortage of water, feed, and occurrence of diseases can adversely affect the performance of animals.

Events and Husbandry	Months											
	D	J	F	M	A	M	J	J	A	S	O	N
Flush feeding								■	■	■		
Mating							■	■	■	■		
Kidding	■	■	■	■							■	■
Lactation/Suckling	■	■	■									
Weaning	■	■	■	■	■							
Lactation/ Commercial milk			■	■	■	■						
Hair cut							■	■	■			
Concentrate feeding	■	■										
Grazing on rangeland			■	■								
Grazing on stubbles							■	■	■			
Dipping						■	■	■	■			
Dry seasons					■	■	■	■	■	■	■	
Wet seasons	■	■	■	■								■

Source: compiled by author

Table 6. Management and husbandry calendar of goats in Jordan.

7. Current and prospects for goat breeding in Jordan

The breeding program for goats in Jordan has not been set up, yet considering breeding objectives, selection criteria, genetic parameter evaluation, etc. Many goat breeds are kept by goat owners throughout the country and few of them use progestagen sponges for estrus synchronization and artificial insemination is no longer undertaken at the farm level. A high proportion of farmers owned crossbred goats as a result of either indiscriminate uncontrolled breeding or controlled upgrading of their does with Damascus bucks. At farm level, goat farmers mainly used subjective more than objective selection criteria to attain their breeding objectives. Farmers' decisions on selection criteria for replacement does of each breed are shown in **Table 7** [10].

In Jordan, goat breeding efforts initiated through the governmental organizations (Ministry of Agriculture, National Center for Agricultural Research and Extension). There are two stations that breed major goat breeds; one located in northern part of Jordan (Al-Kanasry) and the major goat breed raising is Mountain Black breed. The other station located in west-south of Jordan (Al-Waleh) and the major goat breed keeping is Damascus goat. The non-governmental organizations are also involved in goat breeding. For instance, few faculties of agriculture conducting researches on goat breeding aim to increase performance of goats by keeping different goat breeds mainly Bedouin, crossbred, and Damascus.

In Jordan, a national breeding program should be set up aiming to improve the performance, the genetic resources, and productivity of the country's goat population. To achieve this, scientists at research organizations and centers should involve farmers in breeding program and use modern methods that can be used to improve the breeds. Therefore, determining the capacity of certain indigenous goat breeds to cope with climatic change impact may prove a

comparative advantage compared to non-adapted breeds and eventually sustain goat production and conservation of indigenous genotypes.

Selection criterion	Overall
Does source	77
Last season productivity	59
Longevity	52
Disease resistance	49
Overall merit	41
Twinning ability	19
Fertility	13

Source: [10].

Table 7. Overall proportion of goat farmers of different breeds considering doe selection criteria.

8. Goat diseases and health management

Diseases of goats in Jordan are mainly caused by bacteria, viruses, and parasites. As previously mentioned, diseases were the major constraints affecting goat production. Consequently, economic losses are resulted due to decreased production, reproductive inefficiency, and death of animals. In Jordan, there are many infectious, noninfectious diseases and disorders for goat that mainly include subcutaneous and skin diseases, mastitis, emaciation, digestive disorders, reproductive diseases, and respiratory diseases. The last three diseases cause the major problems and economic losses among goat in Jordan.

The major symptom associated with digestive disease is diarrhea. Diarrhea in neonates is most commonly caused by specific bacteria called *E. coli* that secrete an enterotoxin. A previous study by [20] reported that the perinatal and postnatal mortality (abortion, neonatal) accounted for 98.9% of the total losses of goats in the southern Jordan (**Table 4**). Other study by [24] showed that the top ranked diseases affecting small ruminants in the northern area of Jordan were diarrhea, enterotoxaemia, and pneumonia. The high mortalities in young animals can severely affect the farmers' economic returns. Goats also get sick due to diseases of the respiratory system; pneumonia is the common disease in goats.

To protect the goat herd from disease, the health program (e.g., treatment, vaccination, and dipping) should be interconnected with other activities on the farm such as feeding, milking, and breeding programs. Moreover, the surrounding environmental conditions are important considerations in assuring that health program will be effective for flock. Furthermore, there is a need for good veterinarian services to reduce economic losses. And most of all, the prevention is the useful tool for health management.

9. Conclusion

In Jordan, goat production is an integral part of farming systems and plays a significant role for the food security, socioeconomic, and cultural needs of rural households. In the country, goats are valued mainly for meat, and less for milk, skins, and hair. Favoring goat keeping in these regions is attributed to the ease of rearing and efficiency of low-quality roughage utilization. Due to their unique biological and physiological abilities, they can be raised successfully in zones with poor grass vegetation.

Different Jordanian goat breeds have been identified and characterized under different production systems. Damascus (Shami), Mountain Black, Dhaiwi (Bedouin goat), and Desert goats are the main Jordanian goat breeds.

Goat production systems in Jordan changed gradually and shifted from extensive production to systems that involve cropping of arable land. There are several constraints limiting goat production in Jordan. Feed, disease, and drinking water shortages are some of them.

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Chapter 18

The Goat Dairy Sector in Lebanon

Mireille Serhan and Jessy Mattar

Additional information is available at the end of the chapter

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Abstract

Goat farming in Lebanon is an ancestral heritage that may disappear by the overflowing of new technologies; its protection is necessary for the preservation of patrimonial traditions that support our regions. Although goat milk is very nutritious and is considered as an acceptable food in several parts of the country, its production and handling remain a major problem limiting its consumption. In the Mediterranean region, and particularly in Lebanon, goat's milk is becoming increasingly important especially because of the popularity of its products (Darfiyeh, Aricheh, Serdale, Shankleesh, Labneh and Kishk). There is a growing interest in the consumption of the aforementioned typical goat products, which is partly due to the uniqueness of such foods. Their market is expanding; therefore, there is an increasing interest in maintaining the authenticity of these typical products. Considering the limited data available and the latest developments, the purpose of the present chapter is to (1) analyse the current situation of the goat dairy sector in Lebanon, (2) shed the light on the particular manufacturing practices and ripening tools used to yield a variety of artisanal products, and (3) review the attempts of valorisation of milk from goats.

Keywords: milk production, traditional cheeses, quality aspects, valorisation, goat, Lebanon

1. Introduction

Small ruminants in Lebanon contribute to 25% of milk production. Lebanese goat population counts 403,800 animals [1] of which most of it (96.8%) is Baladi breed.

The goat milk sector in Lebanon continues to improve for many years. Although there were the biological, sanitary and socio-economic constraints, milk production has increased from 21.2 (2008) to 34 (2010) thousand tons [1].

It is mainly intended for direct consumption; but it is also processed only into traditional and local dairy products such as Laban [2], Darfiyeh and Aricheh [3], Serdale [3], Shankleesh [4], Labneh [5] and Kishk [6].

Organoleptic and physico-chemical properties of these artisanal cheeses are defined by natural starters as well as a particular method of production passed from generations to generations. The length of the ripening stage of these cheeses and the specialized containers used to do so, contribute much to their appeal and growing interest.

Goat products have a distinctive and relatively strong flavour compared to cow milk products. Technological parameters influence cheese flavour intensity, since the specialized microorganisms that come into play in characterizing the final product vary in intensity in artisanal products versus mass-produced goat cheese products.

The original products market is expanding not only in Lebanon, but in the whole Mediterranean area as well. The trend towards healthy eating and greener food has led to an increase in the economic importance to goat milk products.

While goat artisanal products are regarded as a nutritious dairy option, parts of the country, which mostly include younger generations, still consider these products as unacceptable for consumption because of its production and handling problem. In fact, data of the Ministry of Public Health in Lebanon showed an increase in the number of reported cases of food poisoning. Some of these cases were related to the consumption of artisanal goat cheeses.

A review of current literature integrated in this chapter provides a unique source of the Lebanese goat sector.

2. Goat milk production and yield

According to FAO [7] in 2009, there was 330,000 sheep and 450,000 goats heads in Lebanon According to the Lebanese Center for Agronomic Research and Studies, the goat milk production in Lebanon accounted for more than EUR 54 million in 2014 with a 76% growth per year and a rising consumer demand.

In fact, sheep and goats have always been an integral part of the rural mosaic in Lebanon. According to a recent study by the FAO [8], goats are mainly referred to as the 'Baladi breed' or 'Shami breed' and sheep are of 'Awassi breed' with local traits.

Both sheep and goats are managed under nomadic and semi-nomadic systems, feeding on natural grasslands, crop remnants, and forest types [8].

The seasonality of dairy production is well defined, due to the scarcity of intensive production systems.

Only a small amount of small ruminant milk production is processed into dairy products such as Kishk or Shankleesh to be sold to consumers or distributors. Producers usually sell the largest part of produced goat milk to manufacturers as whole milk. This account to 87.6% of goat milk and 92.4% sheep milk sold.

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According to Khalifat [9], only 10 supermarket chains spread all over the country control over 35% of the Lebanese distribution market, and over 25,000 traditional and modern sales centres also called *Dukkaneh* prevalent in urban areas monopolize the remaining portion of the food distribution market. *Dukkaneh* present a good opportunity for farmers to sell their products beyond their geographic area since they are less demanding when it comes to quality and marketing [10].

Although the production of milk and meat is relatively low, the demand on such products and their by-products remains very high. In fact, the trend in Lebanon is to sell dairy products on the spot in specialized fridges where consumers choose from a variety of native and international variety of cheese or as readily packaged and branded units. Dairy products represent between 12 and 20% of food products total groceries sales, which is a fairly high amount.

This high percentage of dairy sales is in correlation with the major part that dairy occupies in the Lebanese diet with an estimated 189 Kg [11] per capita yearly consumption. Mediterranean diet in general includes dairy product consumption on a regular basis, and can be included in every meal of the day. Correspondingly, Greece for example has 207 Kg per capita and Spain 190 Kg per capita dairy intake. Producers are directly sought after in the weekends and holidays otherwise the sources of purchase are mainly *Dukkaneh* and supermarkets.

According to a previous study by El Balaa et al. [12], small ruminant products are valued by Lebanese consumers, the favoured products being Kishk and Halloumi cheese usually eaten for breakfast, followed by double cream cheese, Akkaoui cheese and Shankleesh. Halloumi, Akkaoui and double cream cheese, they are consumed mostly multiple times per week and the Kishk's frequency of consumption is at least once a week, of course depending on the families' traditions.

In Lebanon, Ministry of Public Health data showed an increase in the number of reported cases of food poisoning, from 43 in 2002 to 373 in 2004. Some of these cases were related to the consumption of homemade cheese.

The local milk production covered in 2005 more than one third of Lebanon consumption needs (in fresh milk equivalent) [13]. The total quantity of locally produced milk increased up to 252,000 tons in 2005 with a 3% increase from 2004. Lebanese cheese imports totalled 32,000 tons in 2002, and full dairy exports amounted to 420 tons in 2002, especially for Akkaoui and Halloumi cheeses.

3. Strengths, weaknesses, opportunities and threats

For many small herders, goats are seen as hardier animals than sheep since they can roam farther searching for food and also have a longer milking season [10].

On the other hand, the continuous increase in food poisoning cases reported around Lebanon has highlighted the need to monitor the manufacturing of food products in order to avoid future health hazards [13]. Other constraints mainly relate to the production systems, farm management skills, health of the herds, milk and dairy product quality and their marketing [1].

Although goat milk is very nutritious and is considered as an acceptable food in several parts of the country, its production and handling remains a major problem limiting its consumption. The dispersed nature of production across the diversity of small farms, small volumes and seasonality of milk production, high ambient temperatures in the summer, poor handling systems, lack of cooling facilities in remote areas, lack of well-organized transportation and communication systems all create a considerable challenge to goat milk production [14]. One of the concerns regarding goat milk by the general public is the perception that goat milk or goat milk products have a 'goaty' flavour because of a long history of widespread negative popular misconception [15] against goats. Well-produced and well-handled goat milk is indistinguishable in taste and odour from good quality cow milk [15]. Milk in general and goat milk in particular have their unique characteristic flavour but not unacceptable smell or odour. Proper handling of milking goats and bucks by separation, good management and hygiene can eliminate the poor attitude by consumers towards goat milk [15].

An opportunity relies in mixing pure breed goats to increase milk production in their offspring. Breeding local goats with imported Shami breed from Cyprus can be an innovative strategy. Shami goats are special breed for milk production, producing an average of 4–5 kg/day on average compared to less than 1 kg/day average for Lebanese breeds [10].

Traditional grazing system with no supplements provided to the cattle is being threatened by the declining winter precipitation and the Syrian crisis [10]. The lack of foraging and limited access to traditional grazing is forcing herders to shift into a semi-intensive system, which consists of daily feed supplements during the grazing period.

4. Goat products-manufacturing practices and ripening tools

4.1. Darfiyeh and Aricheh

Darfiyeh is a semi-hard goat cheese, and the artisanal cheese-making technology originated in Northern Lebanese mountains for many centuries. It is one of the favourite goat cheeses that owe its strong character primarily to its ripening process, using the goatskin. Traditionally, it is manufactured using the raw goat's milk. Cheeses made under these conditions may not have minimal hygiene and sanitary standards needed to obtain consistent product quality [16].

Initially, Ref. [17] concentrated their attention on the hygienic aspects of its manufacture in order to obtain the original cheese with an improved quality. Due to the growing interest in characterization of traditional products, Serhan et al. [18] define the technological features of its production.

4.1.1. Goatskin preparation

Goatskins preparation was reported by Hosri and El Khoury [17]. After slaughtering of the goat, the carcass is fixed by any of its legs, and then the skin is gently removed. The goatskin, preserved in a fresh atmosphere, is subject to an internal salting during one week. After that, the remaining salt is eliminated. The legs are tied, leaving an opening through the neck.

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4.1.2. Darfiyeh cheese manufacturing

In the full processing of Darfiyeh, no starter culture is added, nor $CaCl_2$ solution. The amount of the microbial rennet powder from *Mucor miehei* (Strength 1:150,000) used is variable, but it guarantees a firm coagulation within 60–90 min. Following coagulation, the curd is compacted for the first drainage of the whey, after which it is pressed by hand into the characteristic shape of a parallelepiped (12, 9 and 9 cm). Subsequently, the whey is boiled and raw goat milk is added to coagulate proteins, in order to get the whey cheese or Aricheh. For ripening, Darfiyeh and whey cheeses are introduced inside the goat's skin, which are stored in a natural cellar at 10–12°C [19] (**Figures 1–3**).

4.2. Serdale

It is a non-pasteurized goat cheese, known by many names: 'Serdale', 'Jebnet el Fokhara', or 'Ambariss'[3].

The traditional way to prepare is to introduce the raw goat milk in jars that have a 2 inches hole in the base and let it ferment for a year and the procedure consists of adding raw milk and salt; and removing the whey continually.

Till now, the same procedure is applied, but the milk is placed in big plastic gallons, for economic reasons.

According to the flow chart of production below, the production of 'Serdale' is done following these manufacturing steps:

The milk is collected from the farmers in the village as a first step. Jars are prepared by soaking them for 10–15 days in the whey of the milk so that the walls absorb whey well, and washing them with local olive oil soap known as 'Baladi soap'.

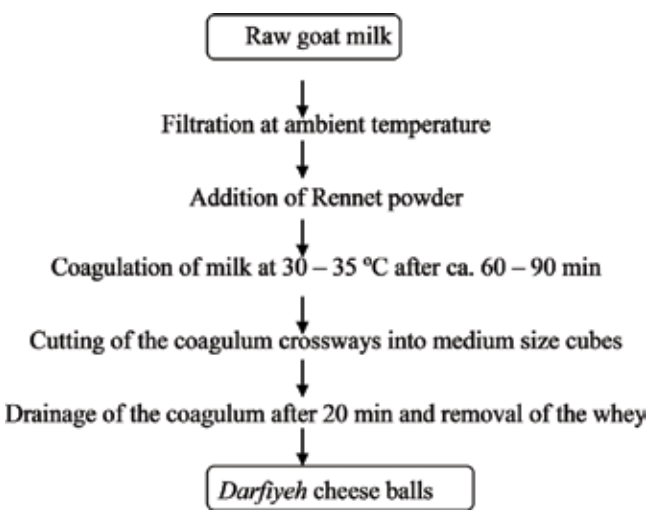

Figure 1. Flow diagram of production of *Darfiyeh* cheese balls [8].

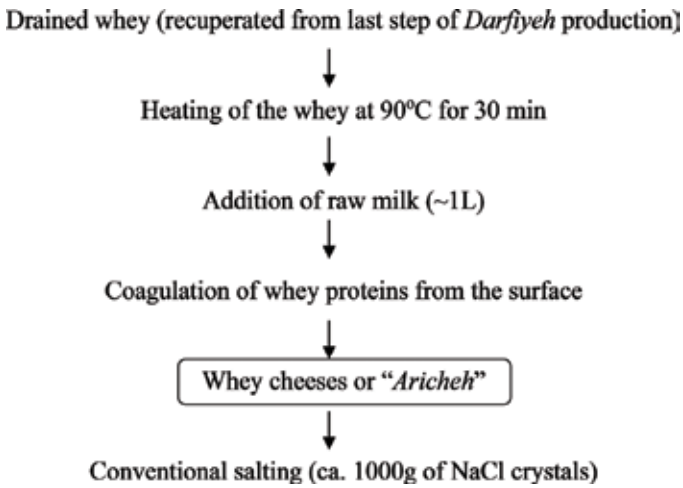

Figure 2. Flow diagram of production of whey cheeses (*Aricheh)* [8].

For every 20 kg of milk, 500 g of salt must be introduced. It is distributed throughout the jar and added in separate amounts periodically. Every time there is appearance of moulds on the surface, it is removed and salt is added again. Total fermentation needs about 15 days to be done. After 15 days, the cap of the jar is removed and the whey is separated from the fermented milk from the whole jar, and raw milk is added again.

This process is repeated several times (about three times) and progressively, until the whey is removed completely by adding raw milk to coagulate the fermented milk.

Finally, the fermented milk is coagulated into small balls and placed in small textile bags to dry to completeness (**Figure 4**).

In this cheese, the coagulation is only lactic without adding rennet, hence there is a need to identify different types of lactic acid bacteria found in milk and goat cheeses and study their effects on fermentation characteristics and cheese.

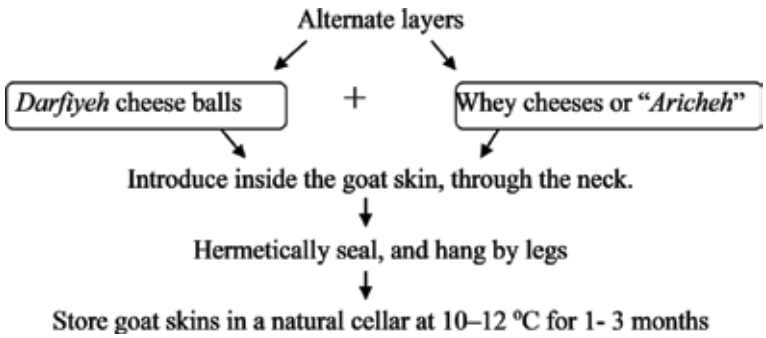

Figure 3. Ripening tools and conditions [8].

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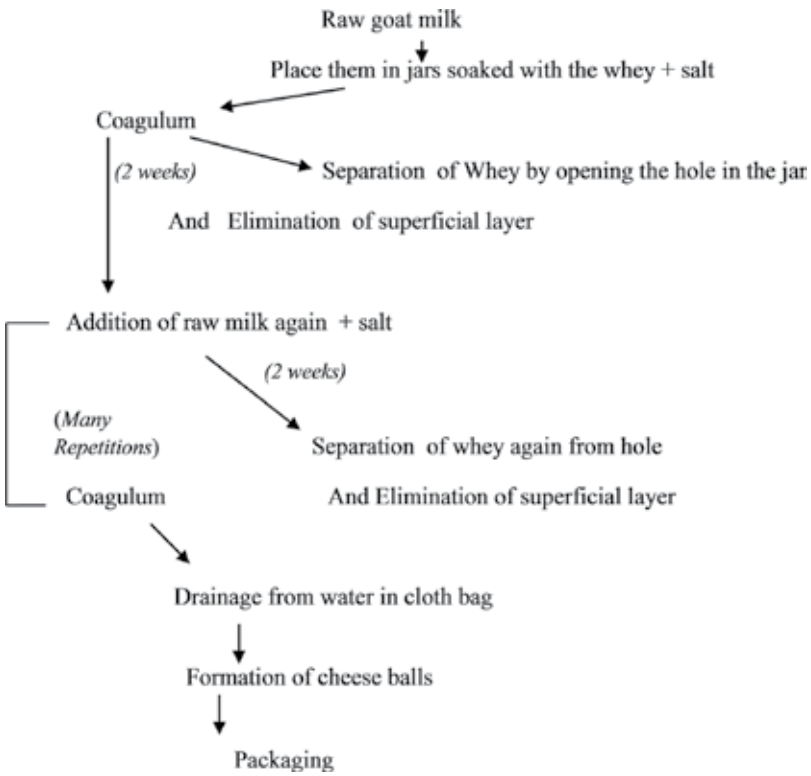

Figure 4. Flow diagram of production of *Serdale* [5].

4.3. Shankleesh

Shankleesh is the only mould-ripened cheese in the Middle East.

Regarding its manufacturing, the precipitate obtained by heating defatted yoghurt is seasoned with salt and powdered with pepper and shaped into balls. The balls are sun-dried, placed in the jars and left to ripen for different intervals of time at ambient temperature.

The mould-covered balls are washed with water, rolled in olive oil and finally covered with powdered thyme (*Thymus vulgaris*). The diversity of microbiota involved during ripening transform the coagulum into a highly flavoured product of unique textural properties.

Furthermore, the low levels of fat (5.6%) [20] coupled with the presumably low water activity of the system and the inhibitory effects of thyme on microbial metabolism confer stability on the product and allow safe storage for long period of time. Although Shankleesh is typically prepared from ewes' milk [20], bovine and caprine Shankleesh products are produced and marketed by local dairy processors, presumably due to seasonal fluctuations in milk supplies.

With reference to the literature available on processing and ripening of different cheese varieties [4, 21, 22], little is known about Shankleesh.

Being coated with thyme, has demonstrated to show inhibitory effects against bacteria. Moreover, its storage in olive oil has made the medium anaerobic thus inhibiting the growth of pathogens. Regardless of the two important advantages, one of the crucial steps in the production of Shankleesh is drying in the open air. At this point, microbial contamination is likely to occur (Zouhairi et al., [23]).

According to Toufeili et al. [4], moderate flavour intensity and well-balanced textural attributes were the salient features of caprine Shankleesh. This was in accordance with the high acceptability scores elicited by the sample, as compared with the bovine and ovine counterparts (**Figure 5**).

4.4. Labneh

Traditionally, Labneh is a fermented milk product widely appreciated and consumed as an important protein source. Different types of milk can be used in the production of Labneh; namely cow, sheep and goat milks, although cow and, to a lesser extent, goat are more common [2]. It has a short shelf life.

With reference to the Lebanese dairy production, Labneh is made from cow's milk (either full fat or skimmed) or from goat milk in more limited availability. It is produced by a traditional old practice by straining milk set yogurt in cloth bags for 12–18 h at refrigeration temperatures, until the desired total solid level is attained. *Streptococcus thermophilus* and *Lb. delbrueckii* sp. *bulgaricus* are the starter cultures used in its production.

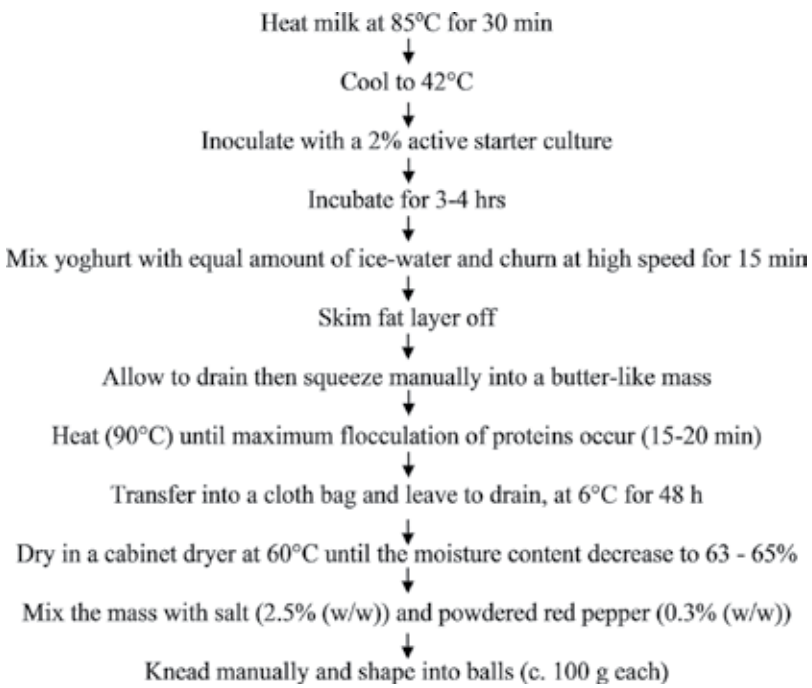

Figure 5. Flow diagram of production of *Shankleesh* [4].

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Modern Labneh manufacturing methods used in industrial scale operations include centrifugation, recombination technology and ultrafiltration.

Popularity of Labneh has led to more interest in its structure and rheological properties [24]. Several methodologies have been utilized in the determination of shelf life of Labneh products by monitoring selected microbiological and physicochemical changes during storage [25, 26]. Preference mapping of commercial bovine Labneh products [27] as well as mapping determinants of purchase intent of Labneh [28] were specifically studied in the Lebanese market.

Moreover, several research studies related to the characterization of bovine or caprine Labneh have been reported in the literature. While cow or goat milk cheeses are classically produced either from 100% of each type, investigations were made on cheeses produced from mixtures of cows' and goats' milk, in the aim of producing a better product under quality characteristics. Considering these aspects, [5] have assessed and evaluated the quality parameters and sensory acceptability of Labneh products made with goats' milk, cows' milk and their mixture, and have compared their quality characteristics with those of Labneh products manufactured with the milk from either cows or goats. The development of such products is an interesting opportunity to produce a goat dairy product that is considered satisfactory by consumers (**Figure 6**).

4.5. Kishk

Kishk is a traditional fermented milk-cereal mixture, widely consumed in Lebanon. It is made from goat's milk, cow's milk or a mixture of both. The product may be manufactured by dairy industries for supermarket retail chains, by granaries or may be made at home.

It is prepared from yogurt, parboiled cracked wheat (Burghol) (ratio of Burghol: yogurt is 1:4) and salt. The ingredients are kneaded daily for up to 6 days at 30–35°C in order to complete the fermentation and conditioning periods. Further to that, the dough is shaped into balls, placed on trays, and dried in the sun for up to 1 week. The dried mixture is milled at granaries. The final dried product is not hygroscopic and can be stored in an open jar for 2 years without any spoilage.

Details of the many different traditional methods employed for the manufacture of Kishk in different countries in the Middle East have been reviewed by several authors. The figure below illustrates the traditional manufacturing stages of Kishk according to Tamime and Robinson [2] (**Figure 7**).

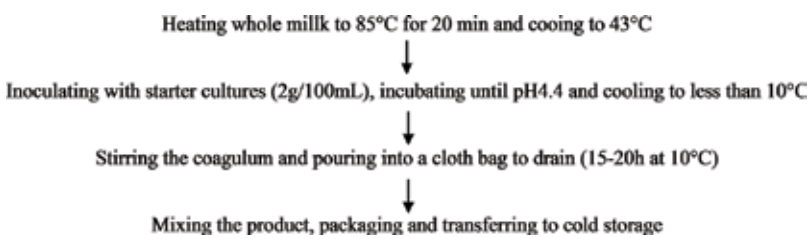

Figure 6. Flow diagram of production of *Labneh* [6].

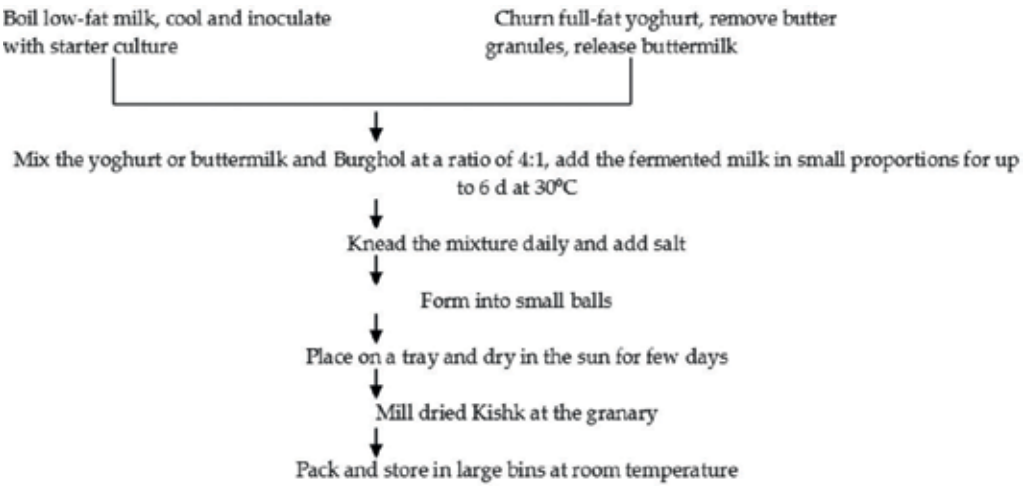

Figure 7. Flow diagram of production of *Kish* (adopted from Tamime and Robinson [2]).

5. Valorisation of milk from goats in Lebanon

5.1. Prospects for protection of dairy products

Artisanal cheeses refer to cheeses prepared by hand using the unique craftsmanship of the cheese maker. They are usually prepared within family of traditional goat farming in Lebanon, which is an ancestral heritage that could disappear by the overflowing of new technologies, and its protection is necessary for the preservation of patrimonial traditions that support Lebanese regions. These products with all particular practices and customs of production appear as a property bequeathed to the Lebanese community, a legacy. Therefore, it is necessary to improve the craft products, built on their cultural property and to give them a symbolic dimension and a specific attention.

5.2. Attempts of safeguarding artisanal products

The technology and composition of most goat cheeses, especially the traditional ones, are not well-documented [29]. The production of traditional goat cheeses is usually carried out on individual farms by shepherds themselves and their families. Most of these cheeses are produced traditionally and manufacturing methods are passed from generation to generation without a technology and regulations standards or pasteurization [30]. Cheeses made under these conditions do not have the minimum hygiene and sanitary guarantees necessary to obtain constant product quality.

The real value of goat cheese is through industrialization under technical and scientific conditions capable of providing products with the indispensable guarantees of quality and constancy [29].

According to the René Moawad Foundation (RMF), Darfiyeh cheese became the first Lebanese artisan produce in the Middle East to receive the 'Presidium' quality level attributed by the

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Slow Food Foundation for Biodiversity (SFFD), an international movement founded in 1996 to protect the pleasures of the table from the homogenization of modern fast food.

The RMF had set a program to support the development of small farmers by providing technical assistance for local milk producers so they can improve the quality of Darfiyeh as well as the production conditions, related mainly to hygiene.

5.3. Protected designation of origin (PDO)

In the European Union (EU), protected geographical indication (PGI), protected designation of origin (PDO) and traditional specialty guaranteed (TSG) seals are used to encourage and protect the reputation for quality of agricultural products and food.

According to the Agriculture and Rural Development, European commission, the following EU schemes encourage diverse agricultural production, protect product names from misuse and imitation and help consumers by giving them information concerning the specific character of the products:

'PDO covers agricultural products and foodstuffs that are produced, processed and prepared in a given geographical area using recognized know-how'.

'PGI covers agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area'.

'TSG highlights traditional character, either in the composition or means of production'.

In 2008, a Swiss-Lebanese Project on geographical indication took place. It has a duration of 3 years. The project aimed at defining an adequate system for the protection of geographical indication in Lebanon, through (1) identifying the products originating from a territory or a region, (2) supporting technically the producers in drafting the legal documents for the registration of geographic indications at the Lebanese Ministry of Economy and Trade and (3) providing the necessary information and working with the supply chain actors on agricultural, organizational and economical aspects of geographical indications.

The Lebanese terroir is very rich in traditional food products. According to a UNDP study, 30% of Lebanese can be categorized as poor. About 10% of the people are rural. Agriculture's share of the gross national product is 6–8% and declining. This decline is negatively affecting the rural poor who still rely on farming for their livelihoods. Subsequently, supporting the implementation of geographical indications, may contribute to enhance the breakdown of traditional food production systems and to reduce the loss of precious indigenous knowledge and the degradation of agro-biodiversity.

6. Safety measures

While it is obvious that the consumer expects the guarantee of high quality, consistent taste, function and the benefits of food safety of cheese, the cheese makers on the other hand want the old style feel to their cheese: all natural, full flavour, and simple packaging. This has

been a major issue in the sales decline of artisanal cheeses. New educated generations are opting for store-bought cheese produced by international manufacturers guarantying safety. However, in the case of artisanal goat cheeses, the desires of both the producer and the consumer can be satisfied if done properly. The key to customer satisfaction depends on focusing on food safety at every step of the cheese manufacturing process without changing the integrity of artisanal cheese-making.

6.1. Poor handling procedures

Most of these cheeses are produced traditionally which means manufacturing methods are passed from generation to generation without a standard technology, standard regulations or pasteurization [30]. Cheeses made under these conditions do not have the minimum hygiene and sanitary guarantees necessary to obtain constant product quality.

Raw goat milk does not undergo any pathogen elimination or reduction step, therefore its safety is mainly dependent on the control of the risk factors that may induce contamination during the cheese-making. Minimizing microbiological hazards that may allow the growth of pathogens by maintaining a hygienic handling and controlling appropriate temperature control during storage and distribution.

Only very few farmers have the innovation capacity to follow the correct milking and handling procedures. Goat milk handling is very primitive with almost no cooling devices for the collected milk, and very poor hygienic conditions linked to the cleaning and disinfection of the utensils, which means poor control over zoonotic diseases. This situation is only true with small holders and poor farmers [8]. The few large holdings with large investment and very modern facilities that exist in Lebanon follow the international norms and standards of milking, handling, hygiene and control of quality.

Brucellosis is quite frequent with some 13% of the livestock affected in 2002, and a high level of infection of humans. Other sanitary problems are also present and have a serious impact on the livestock population. For example, organisms frequently associated with human illness linked to consumption of dairy products are *Campylobacter* spp., pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*.

In the case of a disease-free goat, the routes in which the disease-causing pathogens enter milk are usually through lack of temperature control, or poor farm and personnel sanitation. A correctly run farm therefore, will have milk that is safe to drink right out of the goat [31].

6.2. Good manufacturing practices

The implementation of good manufacturing practices (GMP) during milking and dairy processing in small farms might help them reduce the contamination of dairy products by biological, chemical and physical hazards [32]. GMP defines the series of general measures to be implemented by the food industries to ensure the safety of the process and conformity of food products to precise guidelines.

Moreover, GMP is essential for the implementation of management systems and is the starting point to any food safety system whether Hazard Analysis and Critical Control Point (HACCP), ISO 22000 International Standards Organization, or British Retail Consortium (BRC).

General measures to be implemented by food industries to accomplish with GMP as described by *Codex Alimentarius* include [33]:

- processes necessary for primary production
- design of the premises and equipment
- training, documentation and consumer awareness
- hygiene of the handling personnel
- sanitation and maintenance practices

Although *the Codex Alimentarius* [32, 33] and the Lebanese government may establish general and national guidelines for hygienic practices, the practical implementation to address regulatory requirements procedures adopted consistently by each industry or farm might vary among them. Thus, there is a need to document the practices of each factory in GMP manuals to obtain high quality and safe foods [34].

The implementation of GMPs is a continuous practice based on the management concepts of the plan, do, check and action (PDCA) cycle.

Considering the PDCA cycle, the implementation of GMPs can be divided in four steps: initial diagnosis that includes identifying and addressing safety risks and opportunities, elaboration of road map, addressing of non-conformities and re-evaluation of corrective measures implemented. Initial diagnosis and re-evaluation of corrective measures implemented are usually done by a premises audit visit using a checklist based on the legislation regulating the GMPs in the country. Finally, road maps are generated, based on the previous audit, and corrective measures based on resource priorities and efforts are implemented [34].

Regarding the artisanal cheese-making, implementing the GMPs must be cost effective because of the size of the business is usually of small scale, so several indicators can be used to evaluate the benefits of the implementation of GMPs such as microbiological indicators and perhaps increased appeal to consumers that leads to increased sales.

Typical conditions regarding lack of compliance with GMP in artisanal cheese-making usually include:

Presence of insects and domestic animals as well as unused equipment in the external area, absence of recording thermometers, cracked floor and stone walls, absence of personnel hygiene, food additives in unlabelled containers, use of wooden materials entering into contact with the food, lamps with no protective covers, hoses lying on the floor, personnel not wearing appropriate garments and footwear for working in a food processing plant, and not trained in GMP, and no enforcement of hygienic practices [34].

In addition, production personnel in Lebanon usually gain from past generations of cheese makers, but lack scientific and technological training to realize the consequences of their cheese handling.

Some vital recommendations for the dairy processing industry include [34]:

a. Build strong relationship with milk producers, in case other than themselves, to improve milk quality and quantity, together, in win-win relationships,

b. improve compliance with GMPs after being trained of its principles,

c. train everyone in fundamentals of dairy processing science and technology and

d. when the proper time arrives, adopt HACCP and ISO.

It is also recommended that the dairy processing industry receives training in novel cheese-making technologies.

The existence of a manual describing how GMPs are accomplished by each processing plant is of foremost importance to ensure their continuous evaluation and improvement by processing plants, governments and partners [31].

Therefore, distributing manuals to all artisanal cheese makers seems a fundamental step in educating them about the minimal procedures to follow to guarantee safe cheese. Arabic printed, and loaded with picture should be the adopted format that enables everyone to understand required guidelines.

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Edited by Sándor Kukovics

Goat science covers quite a wide range and varieties of topics, from genetics and breeding, via nutrition, production systems, reproduction, milk and meat production, animal health and parasitism, etc., up to the effects of goat products on human health. In this book, several parts of them are presented within 18 different chapters.

Molecular genetics and genetic improvement of goats are the new approaches of goat development. Several factors affect the passage rate of digesta in goats, but for diet properties, goats are similar to other ruminants. Iodine deficiency in goats could be dangerous. Assisted reproduction techniques have similar importance in goats like in other ruminants. Milk and meat production traits of goats are almost equally important and have significant positive impacts on human health.

Many factors affect the health of goats, heat stress being of increasing importance. Production systems could modify all of the abovementioned characteristics of goats.

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