

*Animal Science, Issues and Professions*

# GOATS

Habitat, Breeding and Management



*Diego E. Garrote* ♦ *Gustavo J. Arede*  
*Editors*

NOVA



**ANIMAL SCIENCE, ISSUES AND PROFESSIONS**

# **GOATS**

## **HABITAT, BREEDING AND MANAGEMENT**

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**ANIMAL SCIENCE, ISSUES AND PROFESSIONS**

**GOATS**  
**HABITAT, BREEDING**  
**AND MANAGEMENT**

**DIEGO E. GARROTE**  
**AND**  
**GUSTAVO J. AREDE**  
**EDITORS**



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## **PREFACE**

In this book, the authors present topical research in the study of the habitat, breeding and management of goats. Topics include the production and commercialization of goat milk and cheese in Northwest Argentina; infectious causes of abortion in goats; the oral environment and diet choices in goats; acute phase proteins as biomarkers of mastitis in dairy goats; and the influence of feeding goats with thyme and rosemary extracts on the physicochemical and sensory quality of cheese and pasteurized milk.

Chapter 1 – The Argentinean goat dairy production is mainly developed in small-scale farming, in a wide variety of situations. There are different types of dairy farms, coincidentally with the greatest diversity of climatic and geographic conditions and according to: goat breed (Saanen, in valleys of the province of Jujuy and Creoles in the Quebrada de Humahuaca), milk production level, type of animal feeding, infrastructure, cheese production and type of farm organization. Each farm produces goat's milk and cheese with characteristics different from others. Goat's cheese is sold in each zone according to the traditions of the place. We evaluated on one side, the business development of productive establishments and on other side, aspects concerning the physicochemical quality of goat milk and cheese, milk admissibility for cheese production and the theoretical yield. We evaluated the characteristics of cheeses taking into account the production cycle and the origin zone. We analyzed the potential and expectations of communities and we identified training needs. We contributed to the acceptance of include a stage of milk pasteurization to ensure quality and cheese safety and to the need of improve process stages. Milk samples were taken from farms and cheeses were elaborated following a traditional scheme of the zone, but using pasteurized goat's milk and ferment developed in the laboratory. Milk and

cheeses were characterized through its chemical composition. We used theoretical equations to predict cheese yield and we compared them with the real one. The goat's milk production cycle has three periods for the valleys and two for the Quebrada de Humahuaca. The main differences in milk composition were found in protein content and fat, but cheese composition shows no differences them. Saanen goat's milk presents higher proteins content from December to February (4-4.5 g/100 g), coincidentally with the rain period and the greater food availability. Lipid content shows a maximum (5.7 g/100g) at the beginning of the same period. Saturated fatty acid: monounsaturated acid relation varies during the cycle. Goat's milk from the Quebrada de Humahuaca (Creole race) shows an increase in composition through the cycle and presents a minimum at the end of the first period. The real yields of process were of 15.27% and 22.96%, for valleys and the Quebrada, respectively. The calcium content is significantly different between both zones. All these variations could be related to the type of food consumed by goats, grazing grasses and shrubs vary by region and can result in different nutritional contributions to the animal. We found that the development level of the organization influences significantly in the production ways, production level and duration of the productive cycle. The aspects surveyed on the basis of the value chain result in the identification of: vision, entailment forms, entrepreneurship and the process of adjudication-taking roles, to obtain the sustainability. This process allowed the rise of interdisciplinary and intercultural teams and the creation of bonds that facilitated achievements altogether and the systematization of the intervention strategies, appropriate to generate productive organizations which allow the sustainable develop of agro-industrial activities.

Chapter 2 – World production of goat's milk is estimated at 15.5 million tons annually, as it is considered a highly prized product it is easily produced on a small areas, also a tool to increase the sustainable profitability of the farmer. Because of the higher digestibility and hypoallergenic properties of goat's milk, this is mainly consumed by the elderly, sick as well as infants. It is estimated that symptoms of cow milk allergy occur between 2.5% to 7% of infants, and research suggests that one third of allergic new-born Infants to cow's milk are tolerant to goat's milk, however often there is confusion between intolerance and milk allergy, mainly caused by alpha-s-1-casein, abundant in cow's milk. Goat's milk has a guaranteed space in the market, due of it is high biological value and low allergenicity, when compared to cow's milk and soya milk. However, there are obstacles to expansion the dairy goat market largely because of: instability in the product offering; cultural resistance

of consumption, with the “goat being the cow of the poor people” and low acceptance due to the typical flavor or poor flock management. The milk and dairy products provide a large portion of saturated fat consumed and some fatty acids that are essential to human nutrition. In the recent past, milk and dairy products have become unpopular between nutritionists, but the bad reputation of saturated fatty acids, should not be widespread, since today it is known that stearic acid (18: 0) has no the atherogenic effects. The essentiality of certain fatty acids has been established by the several researches due to the inability of animals and humans in synthesizing them, and its deficiency causes disorders of growth, changes in the skin, several behavioral disorders, immunological and neurological changes. Comparative studies used as references by medical associations and health institutes for the dietary reference intakes (DRIs), compares the western diets with Greek diet and the Paleolithic period, through simulations based on modern-day hunter-gatherer populations, suggests a ratio of omega 6:3 of 1-2:1, consumption much lower in saturated fatty acids, total fat and almost no of trans fatty acids, except the CLA, being more balanced and healthier than does today’s diet. This is evidenced by lowest rates of cardiovascular disease, cancer, infant mortality and highest life expectancy in the Greek Islands, where the diet consists largely by Mediterranean plants and moderate portions of yoghurt and cheese from goats and sheep reared on pasture. However, care should be taken in causal studies, since the origin of carcinogenesis and cardiovascular diseases is multifactorial. Thus, there is a challenge to improve the ratio of hypocholesterolemic and hypercholesterolemic fatty acids and decrease the atherogenic index to human health, which in the next few years can become a tool in the promotion of production systems of dairy goats based on pasture and supplementation.

Chapter 3 – The occurrence of abortions in goat herds at a level that significantly affects herd performance is a common clinical problem. The microorganisms that cause abortions in goats are viruses, bacteria, and protozoa. The viruses include caprine herpesvirus 1, border disease, and Akabane virus. The bacteria species include *Brucella melitensis*, *Campylobacter* spp, *Chlamydophila abortus*, *Coxiella burnetti*, *Leptospira* spp, *Listeria monocytogenes*, *Mycoplasma* spp, *Salmonella* spp, and *Yersinia pseudotuberculosis*. The protozoa species include *Neospora caninum*, *Sarcocystis* spp, and *Toxoplasma gondii*. Each cause of abortion is discussed in this chapter, including the etiology, the clinical signs, the diagnosis methods, and the management strategies for the disease.

Chapter 4 – There is ample evidence that ruminants are capable of making choices between different foods that provide a more balanced diet that would be obtained by eating at random. In the particular case of goats, they occupy a diversity of habitats and different breeds present variability of feeding behaviors resultant from adaptations to the existent plant species. In their food search activity, individuals are faced with variable amounts of plant secondary metabolites (PSMs), which may present some toxic and anti-nutritional effects depending on the individual's ability to deal with it.

The oral cavity has a key role in the recognition and decision processes of ingestion or rejection. In this chapter we will first consider how goats identify foods and behave according to the food items available. Focus will be done on the importance of taste sense in this process and the information available on the main structures involved in taste detection and perception in goats will be reviewed. In a second section we will focus on the characteristics of goat's saliva, particularly in terms of their protein composition, presenting results obtained by our research team.

Chapter 5 – Mastitis is probably the most costly of the infectious, endemic diseases to affect dairy species. Though most mastitis occurs as a low-grade, subclinical infection, it increases milk leucocyte content, reduces milk production and increases milk bacterial content. These all contribute to reduced milk value as a food and in monetary terms. The prevalence of such infections is also a significant risk to uninfected animals in the herd. The detection level of subclinical infections within a herd is very poor and the currently available tools for diagnosis are of limited use. This is especially so in goats, which have very high normal somatic cell counts because of their apocrine secretory anatomy.

Lately, there has been a lot of excitement about the application of tests for the acute phase proteins (APPs) to monitor animal health. It has been shown that increases in concentration of APPs can be measured in milk of mastitic goats before the appearance of clinical signs. The combination of several APPs with different responses may be used to achieve the highest sensitivity. Moreover, use of these combined APP indices could be employed in monitoring recovery following antibiotic treatment. It is expected that in the near future the development of cheaper automated assays for determination of major APPs and the increase in the number of experimental studies about APPs response will contribute to a wider use of these proteins as biomarkers of the disease. Therefore, their inclusion in the routine biochemical profiles in dairy goats and other species will be of great diagnostic value.

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Chapter 6 – The use of natural antioxidants and flavenoids from by-products derived from aromatic plants can be considered as an alternative to using synthetic antioxidants in the food and pharmaceutical industries. In this sense, feeding goats with aromatic plant byproducts constitutes an interesting option for goat husbandry which increase the quality of the final products while reducing feeding costs. In this chapter feeding goats with *Thymus zygis* spp. and *Rosmarinus officinalis* spp. by-products was studied to determine the influence on the physicochemical composition (dry matter, fat and lactose content), cryoscopic point, somatic cells count of pasteurized goat milk, and on the physicochemical composition, microbiology and sensory analysis of Murcia al Vino cheese as a goat-derived product. In milk significant differences were observed in dry matter, protein and lactose contents while no significant differences were showed in fat, somatic cell count or cryoscopic point. In Murcia al Vino goat cheese no significant differences were determined in any of the physicochemical and microbiological parameters, although significant differences were observed after the sensory analysis, mainly in texture, taste and overall acceptance. The cheese produced with goat milk supplemented with *Thymus zygis* spp by-products showed higher overall acceptance. Both products can be considered of interest for contributing to the range of healthy foods increasingly demanded by consumers.



*Chapter 1*

**PRODUCTION AND COMMERCIALIZATION  
OF GOAT MILK AND CHEESE  
IN NORTHWEST ARGENTINA**

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**ABSTRACT**

The Argentinean goat dairy production is mainly developed in small-scale farming, in a wide variety of situations. There are different types of dairy farms, coincidentally with the greatest diversity of climatic and geographic conditions and according to: goat breed (Saanen, in valleys of the province of Jujuy and Creoles in the Quebrada de Humahuaca), milk production level, type of animal feeding, infrastructure, cheese production and type of farm organization. Each farm produces goat's milk and cheese with characteristics different from others. Goat's cheese is sold in each zone according to the traditions of the place. We evaluated on one side, the business development of productive establishments and on other side, aspects concerning the physicochemical quality of goat milk and cheese, milk admissibility for cheese production and the

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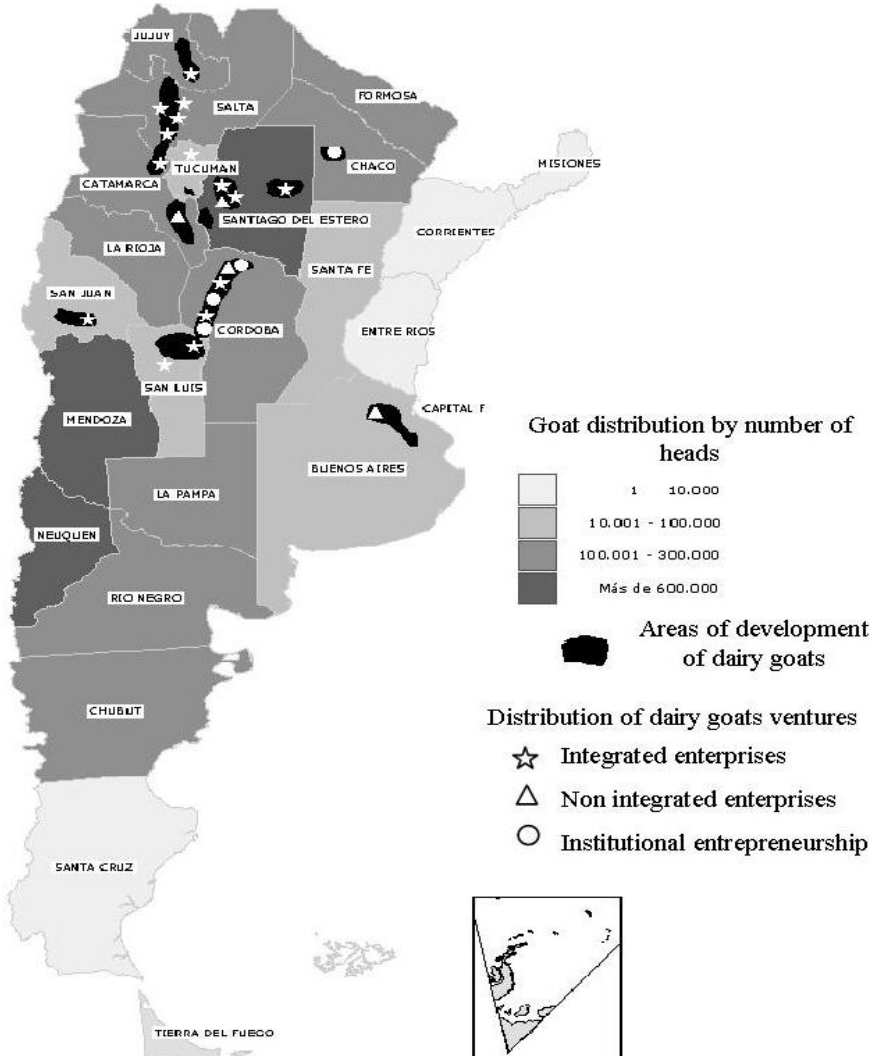
theoretical yield. We evaluated the characteristics of cheeses taking into account the production cycle and the origin zone. We analyzed the potential and expectations of communities and we identified training needs. We contributed to the acceptance to include a stage of milk pasteurization to ensure quality and cheese safety and to the need of improve process stages. Milk samples were taken from farms and cheeses were elaborated following a traditional scheme of the zone, but using pasteurized goat's milk and ferment developed in the laboratory. Milk and cheeses were characterized through its chemical composition. We used theoretical equations to predict cheese yield and we compared them with the real one. The goat's milk production cycle has three periods for the valleys and two for the Quebrada de Humahuaca. The main differences in milk composition were found in protein content and fat, but cheese composition shows no differences them. Saanen goat's milk presents higher proteins content from December to February (4-4.5 g/100 g), coincidentally with the rain period and the greater food availability. Lipid content shows a maximum (5.7 g/100g) at the beginning of the same period. Saturated fatty acid: monounsaturated acid relation varies during the cycle. Goat's milk from the Quebrada de Humahuaca (Creole race) shows an increase in composition through the cycle and presents a minimum at the end of the first period. The real yields of process were of 15.27% and 22.96%, for valleys and the Quebrada, respectively. The calcium content is significantly different between both zones. All these variations could be related to the type of food consumed by goats, grazing grasses and shrubs vary by region and can result in different nutritional contributions to the animal. We found that the development level of the organization influences significantly in the production ways, production level and duration of the productive cycle. The aspects surveyed on the basis of the value chain result in the identification of: vision, entailment forms, entrepreneurship and the process of adjudication-taking roles, to obtain the sustainability. This process allowed the rise of interdisciplinary and intercultural teams and the creation of bonds that facilitated achievements altogether and the systematization of the intervention strategies, appropriate to generate productive organizations which allow the sustainable develop of agro-industrial activities.

## 1. INTRODUCTION

There are dairy goat's establishments with variety of situations in 16 Argentine provinces (Maggio y Lizziero, 1999), as shown in Figure 1. In almost all cases, producers have started in the activity, looking a profitable alternative for their small and medium farms.



In contrast to milk cow production, goat milk production is characterized to be seasonal, with average volumes between 900 and 1200 ml of milk per animal per day. As a result, the daily volume produced in a goats herd national has led to the artisanal industrialization, with small equipments, collecting milk for to concentrate production in one day of the week (SAGPyA, 1998).



Source: CEPAL, 2004.

Figure 1. Distrubtion of dairy goat’s establishment in Argentine.

The Northwest Argentinean (NOA) is part of the central Andean region of South America. The Andean and Sub-Andean valleys, located in the central sector of the Provinces of Jujuy, Salta and Tucumán west, is a mountain area with unfavorable socioeconomic conditions, primarily engaged in intensive irrigated agriculture practiced by small-farmers in their most indigenous descent. These valleys have great advantages over other countries or regions in terms of ecosystems, species, genetic diversity and technological knowledge, which if utilized properly would enable the tenable and sustainable development in the region. The farmers of this area performed horticultural crops, fruit and breeding of small livestock such as sheep and goat. This last represents one of the most important species because of its small size, their high adaptation capacity and docility (Pinto et al, 1992).

Jujuy has great climatic diversity and varied ecosystems: Yungas, Quebrada, Puna and Valleys. The Quebrada region has the largest part of the goat flock in the province. Of national milk production, 0.01% are goat's milk which one 90% goes to making cheese. Jujuy participates with 7% of the production of milk aimed to goat cheese and the 6% of the production goat cheese. These values fall within the six major milk-producing provinces and goat cheese in the country (UIA , 2007).

The milking goat's and making goat cheese for personal consumption or for sale at tourist sites, is usual in the province with the introduction of milk producing breeds as "Saanen" and "Anglo Nubian" in the Valley, although the main breeding traditional is Creole. In the rugged geography of the Quebrada de Humahuaca, inaccessible to other animals, these Creoles goats are bred because is a breed that characterized by its hardy and easy adaptation to the environment. The goat cheese is the most important milk derivative in this region. It is made in traditional way with characteristics and presentations that vary according to the process and customs of each place.

It is important to note that within a species, different breeds produced milk whose composition varies and consequently may have different properties (Alais, 1985, Juárez et al, 1991, Grappin et al, 1981). This variability in the composition is mainly due to genetic and physiological factors such as race, individual characteristics, stage of lactation, handling, climate, composition and type of food. The main differences are in the content of protein and fat (Alais, 1985), which are specifically modified by diet (Grappin et al, 1981). Variations in milk composition directly affect the characteristics of the cheeses (Castagnasso et al, 2007), so the knowledge of the composition of milk is important in assessing product quality and process yield. Cheese yield then will be subject to variations in the percentage of total dry extract, casein and

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calcium added to promote coagulation (Mansur Furtado, 1984; Miceli et al, 2007).

In general, the type of goat's producer in the Quebrada de Humahuaca corresponds to small farmers with the structural problems of its own, as precarious land tenure, little or no capacity of investment, great difficulty in access to credit and deficiencies in infrastructure. Goat production is framed in what is defined as subsistence economies, in which is developed as a complement to other activities. The workforce is familiar and much of what is produced is used for their self-consumption (Martinez et al, 2010).

The people of the Quebrada have an enormous heterogeneity, little development of human and social capital and weak linkages with institutions and market due their socioeconomic and cultural conditions. Exceptionally, some peasant organizations of small producers have adopted schemes of micro and small businesses, generating productive and socio-economic options in rural areas, such as cooperative models, associative and more often family units. The limited commercialization of agricultural production is carried out through intermediaries, at prices that do not cover their costs. These producers require that its activities are profitable enough to cover their basic needs and improve the quality of life for their families. The current income is insufficient, making it necessary give value to their work by incorporating it into the assessment of costs and adding value to primary production through appropriate technological processes. At present, are made isolated attempts of agro-industrial development without incorporation of technology, artisanal level, with a shortage of capital and little chance of access to finance for investment in the sector and without skilled labour.

In recent years, in a search context of productive alternatives, has taken particular interest the development of projects for the production of goat cheeses with and without the incorporation of natural components flavored.

The analysis of the value chain according to Porter (1985) begins with the recognition that each company or business unit is "a series of activities undertaken to design, produce, market, deliver and support their product". The application of this concept allows the decomposition of the agribusiness sector at different levels, ordered from the access to the raw material to the consumer who purchases the product food, including all elaboration process. There will be not adding value if are not guaranteed the factors that make it possible to obtain raw materials and not be will achieve reach markets if the community does not develop skills for sustainability.

On the other hand the importance of entrepreneurship education is critical to the development of a country and especially in the agro-rural industry, so it

must be analyzed in its complexity. Entrepreneurship provides the ability to manage resources in a given context, from to a perspective employer or self-management. This justified the priority to promote and support the development of these skills.

Several diagnoses made from the study of status and situation of micro and small enterprises in rural areas identifies a set of problems that hinder the development of agribusiness, linked to factors of production and the associative capacity (Olarte, 1986). The main limitations are related to the low insertion of the products on the market, the difficulties of organization and business management as well as the lack of legal, financial and technological resources. This situation has been perceived by different organizations, both governmental and non-state who tried to provide support for solutions. However, the activities were carried out in a disjointed form proving insufficient to achieve productive and commercial development in the area. The contributions to the communities were approached according to the capabilities and expertise of each organization: some experts in social organization aspects, others in marketing, but without integration of efforts.

In the eighties of last century originated a school of thought aimed at improving the welfare of the peasantry in Latin America through the appreciation of his own productions: rural agroindustry (RAI), defined as the activity that allows increase and retain, in the rural areas, the added value of production in rural economies, through the execution of tasks in post-harvest in the products from forestry-agricultural farms, such as selection, washing, sorting, storage, preservation, processing, packaging, transportation and marketing (Boucher, 2000).

This concept of rural agribusiness has been discussed, analyzed and modified in light of the environment and the realities of RAI in the field, evolving in terms of socio-economic and political. Today is part of an emerging form of see the rural development so-called "New Rurality" (Echeverri y Rivero, 2002). The rural population in general is poor; one of the reasons for their low income is the lack of development of their agricultural activities associated with its small scale. Its primary products are marketed through intermediaries, obtaining prices that do not reach than cover their costs. The value adding to agricultural production is limited mainly by lack of capital to invest in equipment and technical training. The search for alternatives to adding value and / or the incorporation of new technologies feasible to implement, is not sufficient to initiate a process leading to the creation and sustaining of a business. However, the small rural agribusinesses fill in a gap of market that hardly is covered by big agribusiness, focused on

consumer products and large scale production. For impoverished areas that face harsh conditions as the Quebrada of Jujuy, the articulation of its producers with large-scale agribusiness is impossible in many cases, so the development of small units engaged in processing local products is the only possibility of joining competitively in the urban market. However, one of the main constraints to the development of local's RAI is in the marketing of products. It is possible to recognize a global food scenario in which small farmers can find new niche markets, especially those related to ethnic or exotic products (Rastoin, 1994). In this regard it is important to analyze the factors associated with the changes in the consumer society and which adopting lifestyles according to new needs, which forces consumers to concentrate their preferences for products with different characteristics, for example by a higher nutritional content, storage facilities, storage and preparation, etc. Agro-industrial activity allows labour demand for the various stages of production, representing a chance to work for rural family members, a situation in which women play a fundamental role. Importantly, local food processing is possible if accompanied by technical assistance and advice for creating productive modules with appropriate technology for processing and transformation of primary products.

The chances of developing an entrepreneurial process are associated with multiple context variables such as: cultural and educational aspects, social capital, socio-economic conditions, the structure and dynamics of production, market factors and regulatory conditions (Kantis et al, 2004). Until recently rural agroindustry (RAI) was an unknown sector which was denied social and economic importance, also thought that the peasants producers had no business skills. A strong movement for RAI promotion has developed currently in Latin America, to help small producers and farmers at enhance production and improve their living conditions, thanks to the revenues generated and jobs created.

The organizational structure and purpose of the RAI, especially smaller ones, responds to a number of cultural factors and especially to the logic to survival of family farming. The current challenge for RAI leads to the identification of strategies related to the production, transformation and enhancement of agricultural production, necessary for the insertion in the production chain and the access to new markets; and also with regard to the management natural resources, which is a complex network of relationships between people, products and territories. In a dairy system this involves the product (milk and dairy products), livestock, dairy producers, input suppliers,

intermediaries, consumers, processors located and interconnected in a given territory (Boucher, 2006).

The RAI comprehensive development of goat cheese producers must consider issues related to process, from the physicochemical and microbiological quality of raw material and finished product, until organizational and administrative processes related to social capital, marketing and planning and organization of each aspects of it. That is why this study focuses on two main objectives: the first is aimed at contributing to the knowledge of the characteristics of goat milk and cheese produced in the two main producing areas of northern Argentina, as initial tool to establish procedures adequate production. To do this we assessed the variation in milk composition of Saanen goats (Valleys) and Creole (Quebrada) and cheeses made from them taking into account the variation during a production cycle. We also evaluated the milk admissibility for cheese production and the theoretical yield. The second objective was focused to accompany the Quebrada and Valleys producers to identify strategies to facilitate the application of management tools to all the areas of RAI, for which we evaluated the processes related to the traditional goat production and the ability to modify guidelines related to production and commercial aspects, such as the incorporation of a milk pasteurization step.

## **2. MATERIALS AND METHODS**

### **2.1. Production of Goat Milk and Cheese**

#### ***2.1.1. Milk Production***

Daily milk production (liters / goat / day) was recorded, in both areas, during the period of cheese production. The data were correlated using the statistical program Statgraphics Centurion XV.

#### ***2.1.2. Sampling and Sample Preparation***

Milk was studied from:

- a) A dairy goats in the area of the Valleys, located in General Belgrano Department of Jujuy, Argentina, in which the flock is made up of Saanen race animals, aged between 2 and 5 years. The calving interval is one year and the period of lactation is from May to February.

Feeding the goats is by grazing of local herbs and shrubs, without supplementation.

- b) A goat dairy located in the Quebrada de Humahuaca, in the village of Bárcena, Tumbaya Department, Jujuy, Argentina, with a flock composed of Creoles goats, whose the period of lactation is from October to February. Feeding the goats is by grazing of local herbs and shrubs, without supplementation.

Taking into account the length of lactation period in each case, the start of sampling was performed after 45 days of calving and lasted for 8 months for the sample of the valleys, on the other hand to the sampling in Quebrada beginning from 15 days after the period of colostrum and continued for 4 months.

In both cases the milk was collected by hand milking, performed once a day. Samples were taken from the total produced daily. The milk is filtered, refrigerated (4°C) and transported immediately to the laboratory in stainless steel containers for examination and processing in pilot plant. The milk was heated to a temperature of 20°C with gentle agitation, for complete homogenization before the analysis.

### ***2.1.3. Milk and Whey Characterization***

The evaluation of the composition of the samples was performed every two months since the start of milking until the end of the lactation cycle. We collected about 7.5 litres of milk at each sampling. A total of 500 ml were separated for the characterization of milk, and the remainder was allocated to the production and characterization of cheese.

We assessed the quality of milk for cheese making, determining: pH, titratable acidity (AOAC 947.05), specific density (AOAC 925.22), alcohol test (IDF 48:1969) and reduction of methylene blue (Kirk et al, 1999).

For the analysis of composition we determined: total protein (Kjeldahl method AOAC 955.04), fat (AOAC 922.06 Acid Hydrolysis), total solids (FIL-21: 1962), ash (AOAC 945.46), casein (FIL-20: 1964).

We evaluated the fatty acid profile for which, was performed a derivatization process according to the Spanish UNE 55-037.73 (UNE, 1973).

Simultaneously, we evaluated the chemical composition of the remaining whey with the methods described for milk.

We made three repetitions of each trial and each sample was evaluated in triplicate.

### 2.1.4. Cheese Yield

Cheese yield prediction was carried out by adjusting the obtained composition data from milk, to the equation of Emmons (1991):

$$Y = (F \cdot K_f) + (C \cdot K_c) + \left[ S + M + \frac{M_{fes} \cdot SW}{1 - SW} \right] Y \quad (1)$$

where, Y: Cheese yield (kg cheese/100 kg milk)

F: Fat percentage (kg fat/100 kg milk)

$K_f$ : Conversion factor of fat in the milk to fat in cheese (0,93)

C: Percentage of Casein in milk (kg casein/100 kg cheese)

$K_c$ : Conversion factor of casein in milk to paracaseinate phosphorus and calcium in cheese (including mineral retention, loss of solids and glycomanopeptides in the whey) (1.02)

S: Percentage of Salt (NaCl) added to the cheese (g salt/100 g cheese)

M: Moisture content of cheese (g water/100 g cheese)

$M_{fes}$ : Fraction of water in cheese that can act as a solvent ( $M - 1.04/Y$ )

SW: Percentage of solids in whey, free from fat and casein (0.065)

We selected the mean of each lactation cycle for the comparison between real yield and the value predicted by Equation Emmons (1991): October for the Valleys and December for the Quebrada. We calculated the real yield of this period with the experimental data, using the following equation:

$$Yr(\%) = \frac{m_C}{V_M} \times 100 \quad (2)$$

where, Yr: Percentage of real cheese yield (kg cheese/100 kg milk)

$m_C$ : Cheese mass obtained (kg)

$V_M$ : Volume of milk processed (lt)

### 2.1.5. Production and Characterization of Cheeses

Raw milk was pasteurized at 65°C for 30 minutes before cheese manufacturing. The milk was cooled until reached 38°C and we added lactic culture in the proportion 1% (V/V) performed according Santapaola et al. (2011),



together with 0.02% (w/v)  $\text{CaCl}_2$ . The culture was allowed to act for about 15 minutes until pH decreased by one-tenth, after was added the curd product (Chymax from Chr. Hansen), consisting of 100% quimosine at 50 ml per 100 liters of milk. This was allowed to act for about 30 min. The curd was cut into 1.5 cm cubes and gently agitated for about 5 minutes. The curd was separated from the whey by means of a cloth filter, which was drained until the curd formed a ball. The cheese was then salted and molded in plastic moulds 7 cm tall and 10 cm wide. The cheeses were salted during the molding stage by adding 2% of  $\text{ClNa}$  (common salt), i.e., 2 g of salt per 100 g of curd, in two layers, one at 2 cm from the top and the other at the same distance from the bottom. The unmolded pieces were placed in a refrigerator at a constant temperature of  $10^\circ\text{C}$  and 85% relative moisture for 7 days.

Cheeses were chemically characterized through the determination of: total protein (Kjeldahl method AOAC 955.04), fat (AOAC 922.06 Acid Hydrolysis), ash (AOAC 968.08), moisture (AOAC 935.29) in a vacuum oven (Shel lab, model 1410) at  $60 \pm 1^\circ\text{C}$  and 25 inch of mercury until constant weight, total carbohydrate by difference, calcium was determined as oxalate by titration with permanganate (Pearson, 1981). There were three repetitions of each trial and each sample was evaluated in triplicate.

#### ***2.1.6. Statistics Analysis***

All results were analyzed using the LSD test, measuring the least significant difference between means with a confidence level of 95%. The analysis was performed using Statgraphics Centurion XV software.

## **2.2. Organization and Management**

After making the diagnosis of both areas, we worked in Quebrada area, considered the least favourable and less developed. We searched strategies relevant for productive contextually, that could provide the means to achieve the transformation of traditional enterprises in RAI; simple tools were used, based on observation, detection of strengths, weaknesses, expectations of the communities as well as training and technical support.

We conducted field visits, meetings and workshops with rural producers in the region to assess the initial situation, expectations and opportunities for development. Different interventions were always previously agreed in order to ensure a high percentage of participation.

We worked with producers in the generation of value chain, thinking in a product quality and negotiable. The analysis of the value chain was carried out using intervention strategies. To start the work discusses aspects that may provide opportunities for link with the community: applied “ice-breaker” techniques to build trust and stimulate dialogue. We identified the inter-relationships of the community with their context.

The producers discussed the construction of a common vision, starting from the skills of group for entrepreneurship.

The enterprising capacity was evaluated from the capacity, availability and resources, applying the value chain. We evaluated the type and level of development of the principal activity or primary, and support activity or secondary, in order to identify points of vulnerability.

We determined the participation in productive activities, broken down by gender and education level, being important factors and determinants for entrepreneurship and we analyzed the activities and roles in relation to raw material production, processing, marketing, technology and equipment available and management.

The availability of raw materials, processing techniques and methodologies, its relation to quality and safety of products, detecting and analyzing the critical points were studied.

We assessed the sustainability of the activity, taking into account the availability factors and capacity of human capital and used strategic tools that provide the SWOT matrix allowing analyzing the internal and external elements in milk production of the Quebrada, starting from an initial situation analysis. We analyzed the tradition in commercialization and marketing used by producers, the adequacy of the organization and infrastructure.

### **3. RESULTS AND DISCUSSION**

#### **3.1. Production of Goat Milk and Cheese**

##### ***3.1.1. Milk Production***

The milk production of Saanen goats during the lactation cycle evaluated is shown in Figure 2. Each point represents a monthly average of the entire herd, calculated from the daily value of milk produced by each goat. It is observed that the lactation cycle is divided into four periods according to the level of milk production: 1<sup>st</sup> period: up to 90 days (from May to July), 2<sup>nd</sup> period: 90-180 days (July to October), the 3<sup>rd</sup> period: 180 to 240 days (October

to December) and the 4<sup>th</sup> period of 240 to 300 days (December to February). Importantly, although in this area does not supplement the diet of goats, have natural food availability throughout the cycle (10 months).

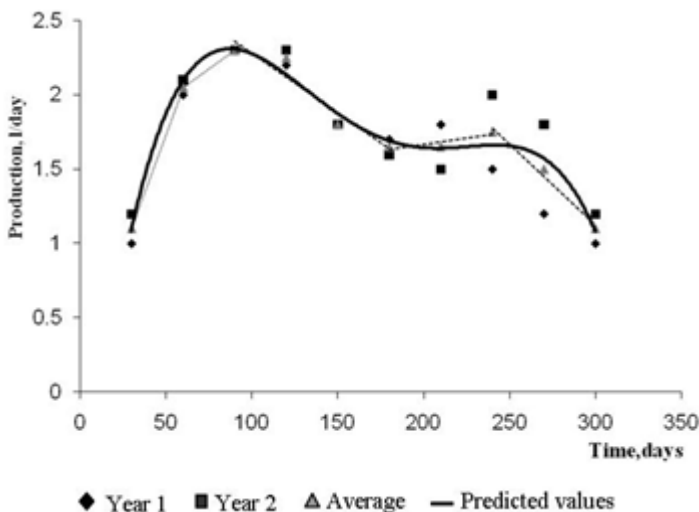


Figure 2. Annual milk production of the Valleys.

It can be seen that the higher milk production occurs during the first three months of lactation, then decreases and remains constant for about four months, from December we can see a sharp drop in milk production until the end of lactation, which is related to the lack of service planning and therefore, calving.

Milk production data were adjusted with 97% confidence, to a polynomial equation of 4th order of type:

$$y = a t^4 + b t^3 + c t^2 + d t + e$$

where, the coefficients takes the following values: a:  $-6 \cdot 10^{-9}$ , b:  $4 \cdot 10^{-6}$ , c:  $-0,0011$ , d:  $0.1061$  and e:  $-1.2375$ .

Milk production in the Quebrada is presented in Figure 3, which shows a continuous decline of production, at high speed in the first section of the curve up 20 days after which time it reaches a value of 65 ml/day/goat; after that the milk production remains constant until the end of the cycle. The milk collection begins in October and the cycle ends in February. The maximum value of milk production was achieved after two days of the milking start, with 279 ml.

The continued decline in production was also observed by Perez et al. (1993) in Chilean native goats.

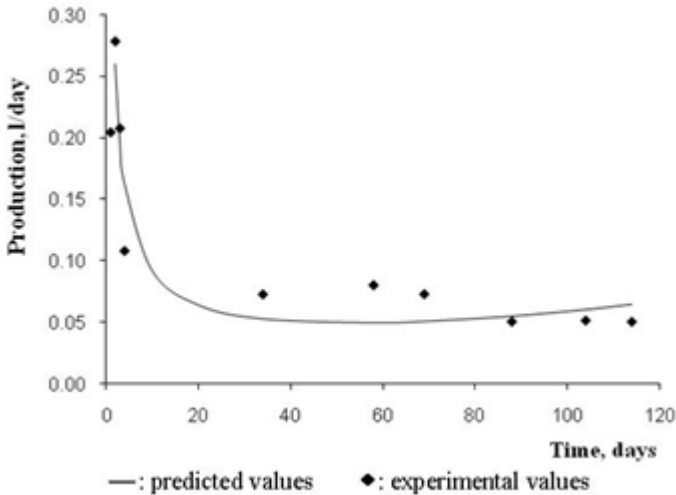


Figure 3. Milk production from goats of the Quebrada.

The significant difference in the level of production between the two areas could be to the Quebrada handling style where the breeding is not separated from the mother (what happened during the course of this study), so the daily collection shown in Figure 3 represents the remaining milk after feeding the breeding (breastfeeding).

The experimental data of Creole goat milk production in the Quebrada, during the cycle studied, adjusted with 95% confidence to incomplete gamma equation of the form:

$$y = a t^b e^{-ct}$$

where,  $a: 0,41 \pm 0,09$ ;  $b: -0,71 \pm 0,22$  y  $c: -0,013 \pm 0,009$ .

This fit is similar to that reported by Leon et al. (2007) to study the dairy goat breed of Murciano-Granadine in Granada. According to these results the milk production cycle of the Quebrada is composed of two periods: the first, up to 20 days of production and second, extends from 20 to 120 days.

Comparing the two areas we can affirm that the cycle length is much greater in the Valley (10 months) than in the Quebrada (4 months) and maxi-

mum milk production (available for processing by-products) of the Valleys area is 8 times greater than that of Quebrada and is approximately 30 times higher for the remainder of the cycle. This accounts for the differences in the availability of water and food and differences in handling goats. The inclusion of management tools in the goat handling contribute to achieving that the goats arrive in good condition at the time of greatest quantity and quality of food.

### 3.1.2. Characterization of Milk and Whey

The physicochemical characterization of the goat milk samples of the Valley and Quebrada are shown in Tables 1 and 2, respectively.

**Table 1. Physicochemical parameters of Saanen goat milk of the Valleys (mean  $\pm$  confidence limit)**

	<b>July</b>	<b>October</b>	<b>December</b>	<b>February</b>
Ph	6.59 $\pm$ 0.01 <sup>(a)</sup>	6.71 $\pm$ 0.01 <sup>(b)</sup>	6.60 $\pm$ 0.01 <sup>(a)</sup>	6.79 $\pm$ 0.01 <sup>(c)</sup>
Density (g/ml)	1.03 $\pm$ 0.01 <sup>(a)</sup>	1.02 $\pm$ 0.01 <sup>(b)</sup>	1.02 $\pm$ 0.01 <sup>(b)</sup>	1.02 $\pm$ 0.01 <sup>(b)</sup>
Acidity ( $^{\circ}$ D)	13.3 $\pm$ 0.1 <sup>(ab)</sup>	13.7 $\pm$ 0.1 <sup>(b)</sup>	14.4 $\pm$ 0.2 <sup>(c)</sup>	12.9 $\pm$ 0.2 <sup>(a)</sup>
Reduction of methylene blue	Good	Good	Good	Good

Means with same letters in the same row were not significantly different ( $P < 0.05$ ).

**Table 2. Physicochemical parameters of Creoles goat milk of the Quebrada (mean  $\pm$  confidence limit)**

	<b>October</b>	<b>November</b>	<b>December</b>
pH	6.77 $\pm$ 0.03 <sup>(a)</sup>	6.71 $\pm$ 0.03 <sup>(ab)</sup>	6.63 $\pm$ 0.03 <sup>(b)</sup>
Density (g/ml)	1.03 $\pm$ 0.03 <sup>(a)</sup>	1.03 $\pm$ 0.03 <sup>(a)</sup>	1.03 $\pm$ 0.03 <sup>(a)</sup>
Acidity ( $^{\circ}$ D)	13.99 $\pm$ 0.03 <sup>(a)</sup>	14.22 $\pm$ 0.03 <sup>(b)</sup>	16.66 $\pm$ 0.03 <sup>(c)</sup>
Reduction of methylene blue	Good	Good	Good

Means with same letters in the same row were not significantly different ( $P < 0.05$ ).

Valleys milk showed significant differences between periods with respect to pH and acidity, this could be due to physiological changes experienced by the goat after calving and by seasonal change, hence the variation of temperature that promotes microbial growth and is also related to the type and availability of food for goats. From the analysis of these results it is inferred

that the samples have been handled in a sanitary condition during evaluated cycle and would not have ruptured fat globules. The methylene blue test gave a clearing time over 5 hours, indicating good quality milk with low microbial content. pH values are within the range recommended by Buxadé (1998) for goat herds. From October the density remained constant and with values lower than those reported by Chacon Villalobos et al (2005).

Table 2 shows the results of the characterization of native goat's milk of the Quebrada. We selected as representative of the first period the October month and two months to the second period: November and December, to assess the seasonal influence on the composition. During the months studied, we found significant differences in the values of acidity, while pH values showed similarity between October and November and between the latter and December. It is observed that pH values decrease throughout the cycle as the acidity increases. This could be attributed to growing of micro-biota due the increase of the temperature associated with seasonal change. The values of density, pH and titratable acidity are within the ranges reported by Chacon Villalobos et al (2005) and Ludeña et al (2006) in Creole goat milk of Perú. The acidity was similar to the values reported by Fonseca et al (2006) and lower than Miceli et al (2007) reports for Creole goat milk, with dietary supplementation.

The study of these physicochemical parameters in both areas shows the suitability of goat milk for cheese making and their importance to infer the hygienic-sanitary quality of milk. The pH values of both areas are between 6.6 - 6.8, acidity between 13°D - 17°D and the density between 1.02 – 1.03 g/ml. These values can be taken as acceptable when evaluating the quality of goat milk in the province of Jujuy, considering that with these milks was possible to develop a curd with suitable characteristics and get cheese physicochemical, microbiological and sensorial acceptable.

Figures 4 and 5 show the contents of protein, casein and fat, in the months studied for the Valleys and Quebrada areas, respectively.

For the Valleys areas (Figure 4), the values of proteins and caseins, show significant differences at the beginning and end of cycle. The fat content was different during the whole study, showing a decrease during the winter-spring seasonal change (July-October). We observed an increase, when the rainy season starts (December) and therefore with increasing variety and availability of food, and a decrease at the end of the cycle, the latter could be due to a dilution factor of the milk. The percentages of fat and protein are within the range reported by Alsina et al (2002), Albanell et al (1999), Salvador et al (2006) and are lower than those reported by Oliszewski et al (2002). Casein

values were higher than those presented by Quiles et al (1990), Salvador et al (2006), Chiatti et al (2006) and St-Gelais et al (1999). The total mineral content, expressed as ash were  $0.84 \pm 0.01$  g/100g in July,  $0.75 \pm 0.01$  g/100g in October,  $0.86 \pm 0.01$  g/100g in December and  $0.87 \pm 0.01$  g/100g in February, they did not differ significant. The total solids for the months of July, October, December and February was  $14.4 \pm 0.2$  g/100g,  $13.3 \pm 0.1$  g/100g,  $13.2 \pm 0.1$  g/100g and  $11.5 \pm 0.1$  g/100g, respectively, and are similar to those reported by Albanell et al (1999), Salvador et al (2006), Alsina et al (2002) and Ramos and Juarez (1993). We can see a decrease in total solids content, according as lactation cycle progresses, producing significant differences between October-December and February.

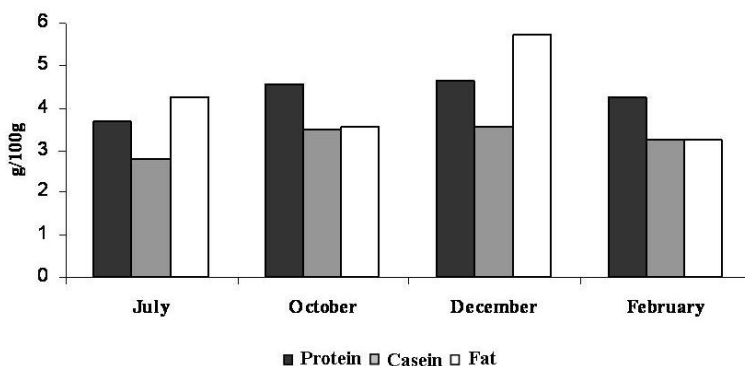


Figure 4. Evolution of the components of goat milk of the Valleys.

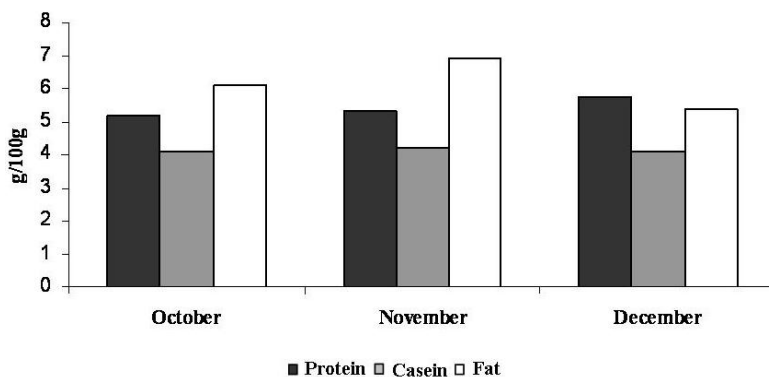


Figure 5. Evolution of the components of goat milk of the Quebrada.

In the case of milk from the Quebrada (Figure 5), the fat content of goat milk showed significant differences in the months evaluated, while proteins differ only at the end of the cycle and casein showed no significant differences in the three months selected in the cycle. The physicochemical parameters mentioned are within the ranges reported by Salvador et al (2005), Oliszewski et al (2002), Paez et al (1996) for goat milk crossbreeding Creole x Anglo-Nubian and Rossanigo et al (1995) in Creole goat milk kept under semi-intensive feeding, but were higher than those reported by Alsina et al (2002).

As total solids results were  $14.5 \pm 0.3$  g/100g for October,  $16.2 \pm 0.4$  g/100g in November and  $17.5 \pm 0.3$  g/100g in December, showing significant differences in the first month of study. The values obtained were higher than those reported by Miceli et al (2007) for the average lactation cycle of native goats and other crossbreeding Creole x Anglo-Nubian and by Alsina et al (2002) in Creole goat milk during the first 105 days of lactation. The ash contents were  $0.81 \pm 0.01$  g/100g,  $0.84 \pm 0.01$  g/100g and  $0.94 \pm 0.01$  g/100g, and by calcium,  $132 \pm 7$  mg/100g,  $126 \pm 2$  mg/100g and  $146 \pm 4$  mg/100g, for the months of October, December and February, respectively, with significant differences in the last month. With regard to ash content and calcium, are within expressed by Medina Espinoza (2003), Chacon Villalobos et al (2005) and Castagnasso et al (2007).

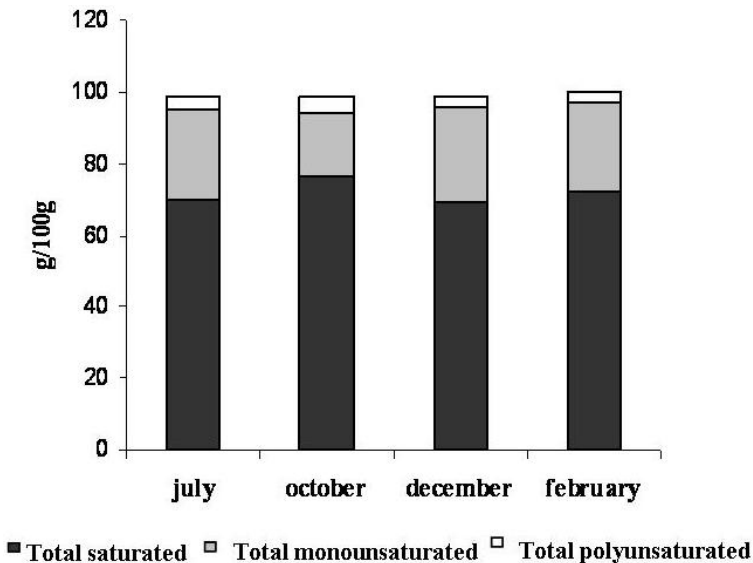


Figure 6. Fatty acid content in goat's milk of the Valleys.



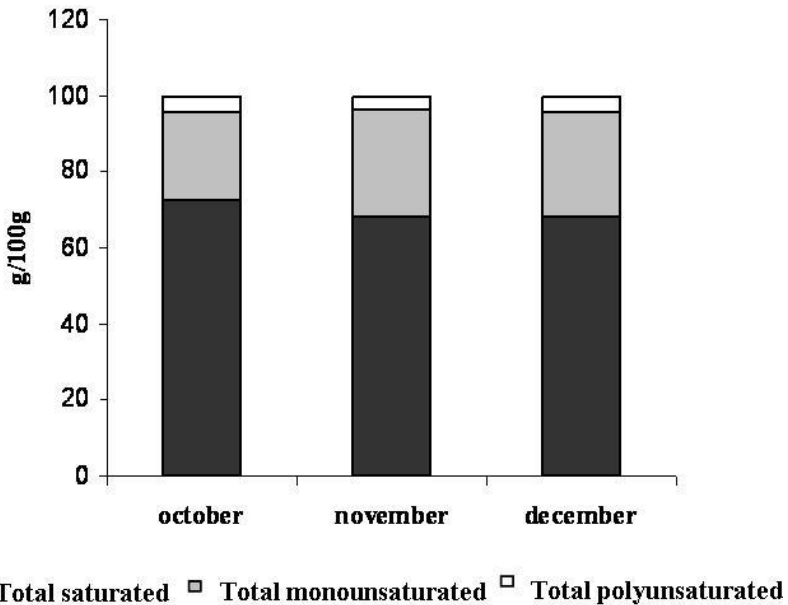


Figure 7. Fatty acid content in goat's milk of the Quebrada.

The fatty acid profiles for both areas are shown in Figures 6 and 7. The fatty acid composition throughout the time of production shows a change in the relationship saturated fatty acids: monounsaturated, which can be attributed to variations in the quality of animal feed, which was observed in both areas.

The highest nutrient content was obtained in the month of December and early summer coinciding with the rainy season, for both areas. Milk from the Quebrada has more fat and casein than milk of the Valleys.

In the Quebrada, the major differences in composition occur in the month of December, while in the Valleys area has been observed that the protein content as casein and fat, increase from July to December, presenting the lowest values in July, which is associated with animal management style that involves nutritional deficiencies in the goat during the cold weather, due to poor food. The nutritional status is improved with increasing availability of natural pastures in the month of November because the goats are fed on pasture without supplementation or supplement their diets. The highest values are obtained in December, to decrease in February, at the end of lactation. Similar differences were also reported by Alsina et al (2002). During the winter months, food shortage affects the nutritional status of goats.

**Table 3. Composition of goat cheese from milk of the Valleys**

Period	Mean $\pm$ Confidence Limit				
	Moisture	Protein	Fat	Ash	Calcium
	g/100 g				mg/100 g
1°	43.3 $\pm$ 0.5 <sup>(a)</sup>	19.5 $\pm$ 0.5 <sup>(a)</sup>	28.81 $\pm$ 0.01 <sup>(a)</sup>	3.65 $\pm$ 0.01 <sup>(a)</sup>	802 $\pm$ 1 <sup>(a)</sup>
2°	51.1 $\pm$ 0.4 <sup>(b)</sup>	20.1 $\pm$ 0.2 <sup>(a)</sup>	22.5 $\pm$ 0.3 <sup>(b)</sup>	3.67 $\pm$ 0.02 <sup>(a)</sup>	851 $\pm$ 1 <sup>(b)</sup>
3°	50.9 $\pm$ 0.5 <sup>(b)</sup>	20.0 $\pm$ 0.1 <sup>(a)</sup>	22.5 $\pm$ 0.3 <sup>(b)</sup>	4.4 $\pm$ 0.4 <sup>(a)</sup>	835 $\pm$ 9 <sup>(b)</sup>
4°	40.7 $\pm$ 0.2 <sup>(c)</sup>	21.4 $\pm$ 0.2 <sup>(b)</sup>	30.4 $\pm$ 0.5 <sup>(c)</sup>	3.69 $\pm$ 0.01 <sup>(a)</sup>	789 $\pm$ 1 <sup>(a)</sup>

Means with same letters in the same column were not significantly different ( $P < 0.05$ ).

It is necessary take to account, that a system that consider producing throughout the year should minimize the variation in the chemical composition of milk and maintain consistent quality and production of cheese throughout the year (Chacon Villalobos et al, 2005).

The compositions of the whey from the cheese process did not differ significantly between the two areas studied. The composition of whey for the month of October (g/100 g) (mean  $\pm$  confidence limit), was: 0.83  $\pm$  0.01 for fat, 1.09  $\pm$  0.06 for protein, 8.09  $\pm$  0.07 for total solids and 0.46  $\pm$  0.03 for casein. The protein content is within the range of values reported by Abaigar (2005) for acid whey from fresh goat cheese soft paste; instead, data of total solids and fat are higher than those reported by the same author. As for casein, the value was near 13% of the total protein, expected value of bovine milk whey according to Inda Cunningham (2000), reflecting an effective enzymatic action of rennet, since most of the casein constitutes the resulting cheese.

### **3.1.3. Preparation and Characterization of Cheese**

The results obtained for cheeses made from goat's milk of the valleys and analyzed to the end of each period, according to the specification of 2.1.5 are shown in Table 3. It is noted that the 2<sup>nd</sup> and 3<sup>rd</sup> periods shown statistically similar composition, while the last period of the cycle is the one with greater differences.

According to the values of fat, cheese from the valleys obtained in the 2<sup>nd</sup> and 3<sup>rd</sup> period can be labeled as "low-fat" but in the 1<sup>st</sup> and 4<sup>th</sup> period cheeses are obtained "half-fat," according to the *Código Alimentario Argentino* (CAA). This leads us to infer the need to include a stage of milk standardization, in order to maintain the characteristics and quality of cheeses constant throughout

the cycle. Taking into account the moisture content and according to the CAA, are soft cheeses.

Greater moisture retention observed in the 2<sup>nd</sup> and 3<sup>rd</sup> periods can be explained because in these periods the milk showed a maximum in casein content that is related to the ability of caseins to hold water in the cheese, coincidentally with Inda Cunningham (2000). On the other hand we must consider that in normal conditions, the gel formed at the end of the coagulation of milk has different ability to stop syneresis whey, as was observed by Vertalet-Guzman (1989).

The composition mean values obtained at final each period of the cycle are within the values reported by Fekadu et al(2005) for goat milk cheeses Alpina; however, were higher than those reported by Mas Mayoral et al (1991) for goat cheese Ibores and Galina et al (2007) for goat cheese crossbreeding Alpina x Anglo-Nubian and lower than reported by Miceli et al (2007) in goat's milk cheeses of Creoles and crossbreeding Creole x Anglo-Nubian.

The fat content of cheeses showed significant differences in the 1<sup>st</sup> and 4<sup>th</sup> periods. In October and December we obtained equal lipid content and were evidence a decrease in the fat content compared to the other periods, so the values do not vary in parallel with the lipid content in milk. The calcium content in cheese was similar to that reported by FAO (2002) for Mexican cheeses. It highlights the contribution of calcium from the goat cheese for indicated amount of 800 mg for children aged four to six years according to the Recommended Dietary Allowance (RDA) by the National Academy of Science (USA, 1989), according to which these cheeses represent an excellent source of calcium for children in the area of production, coinciding with the expressed by Alsina et al (2001).

The moisture average values of Creole goat cheese of the Quebrada:  $52.7 \pm 0.7$  g/100 g correspond to soft cheeses (CAA) and according to lipid content ( $22.8 \pm 0.4$  g / 100g), low-fat cheeses (CAA). The average composition presented similar moisture values reported by Pino et al (2005) for soft goat cheese and lesser than that reported by Galina et al (2007) for goat cheese crossbreeding Alpina x Anglo-Saanem. Protein content:  $20.2 \pm 0.2$  g/100g was higher than results exposed by Pino (2005) and Galina (2007), is also greater than the reports by Mas Mayoral et al (1991), while the fat content reported by the last author is higher than cheese of the Quebrada. Ash:  $3.1 \pm 0.3$  g/100g were higher than the values reported by Galina et al (2007).

The calcium content of cheese made with goat milk Creole ( $544 \pm 3$  mg/100g) was similar to values found by Almenara et al (2007) for fresh goat cheese, from the Spanish Majorero variety and as reported by Ledesma et al

(2007) for the goat cheese variety Palmero. We observed differences in calcium content between the areas studied, this could be attributed to differences in calcium content in milk, caused by the dissimilar feeding the goats, they consume during grasses grazing and shrubs in the region that may result in different nutritional intake.

#### **3.1.4. Cheese Yield**

The Valley milk yields calculated from the Emmons equation (1) were  $15.13 \pm 0.03\%$ ,  $15.69 \pm 0.07\%$ ,  $20.32 \pm 0.02\%$   $11.59 \pm 0.01\%$  for July, October, December and February, respectively. The variation in the theoretical yield is consistent with the variation in milk composition because Emmons's equation takes into account the amount of salt, whey solids and moisture, and mainly the relative contribution of fat and casein, considered the main component responsible for the cheese yield, coinciding with Cozzano and Delgado (2003).

In October, the real yield of the process, obtained from equation (2) was  $15.3 \pm 0.6\%$  and was statistically similar to the theoretical yield of that month, so it follows that can be used Emmons equation to predict the yield cheese. The October performance was similar to that reported by Pierre et al (1998) in European goat's cheeses, two days matured and is expressed in Oliszewski et al (2002) in Creole goat's cheese and superior to those presented by Duquesne et al (1999) in soft cheese and Cosentino et al (1999).

The theoretical cheese yield of milk from Quebrada for the months of October, November and December was 23.37%, 25.55% and 21.89%, respectively. This variation was mainly attributable to the compositional variation of milk leading to productive differences that affect the profitability of the activity. The average real yield obtained for the months of study was  $22.96 \pm 0.03$ . The cheese yield of milk from Quebrada was higher than the values reported by Oliszewski et al. (2002), Duquesne et al. (1999) and Cosentino et al. (1999). These results reflect the behavior of casein as responsible for forming the structure/matrix of cheese, which retains the fat and moisture, coinciding with Cozzano and Delgado (2003).

## **3.2. Organization and Management**

In the area of the valley, there is a single production facility using about 50% of installed capacity, working sporadically in two shifts of operation, producing goat cheese and *quesillo* of milk cow, made with self-milk and

acquired from other producers of the area. This establishment makes its products with pasteurized milk and adding commercial ferment, usually used for making cheese from cow. Also, performs basic routine analysis to determine the quality of the milk used as raw material: acidity, pH and density without microbiological quality control. However, should be clarified that there are no standards for rating the quality of raw goat's milk because the Argentinean legislation does not contemplate. The infrastructure that account is adapted to the needs, enabling the development of differentiated products, with packaging according to the demands of the markets.

We detected the possibility of marketing in MERCOSUR region, as an opportunity being that the products are developed with appropriate technology. This also enables the development of new products that may have funding.

The producers are supported to promote and market their products in national and international fairs. We detected large potential to build a cluster and to access the market while maintaining quantity, quality and getting a good price, if they properly handle costs.

In the area of Quebrada, goat feeding is performed by heterogeneous vegetation grazing limited to species with low productivity, without receiving any dietary supplement and there is no basic information about the nutritional value of their diets. Sanitation practices are common. There is a low productivity of goats, which has been observed in other Andean regions mainly due to nutritional causes (García Gonzalez et al., 2008). They have developed, over time, experience in the management of community troops, the milking by hand. The corrals and the facilities are poor and in some cases non-existent, being exposed to natural predators such as pumas, foxes and even dogs, caused periodic losses. They have no potable water, gas or alternative energy. Producers do not perform an analysis of the total costs of production of milk and cheese, for example do not take into account the cost of labor, the cost of electricity or the cost of raw materials, among others.

Due to weather conditions in the area, large daily thermal amplitudes and strong winds, and low availability of pasture and water during the winter there is a seasonal volume of milk suitable for cheese production in the period from October in late February, coinciding with the rainy season.

The cheeses developed in the Quebrada, are sold directly at the site of production as a traditional craft products made from unpasteurized milk and without addition of commercial ferment, simply taking advantage of the indigenous micro-biota of the milk with the benefits and risks that implies. There has been no quality control of milk used for processing and has not developed a product packaging; cheeses are directly exposed to the environ-

ment, on the edge of the routes and fairs and local markets. This brings with it that can cause cross contamination of the cheese.

The peasant farmers have no access to credit lines due to ignorance of the programs, difficulty in providing collateral and lack of community organization to improve their production scale. This is also the reason that not it allows them to reach other markets.

We detected a high resistance to change by the lack of entrepreneurial vision. The producers are accustomed to self-sustenance due to cultural reasons. They do not consider the needing of inversion in special equipment for processing, due their poverty, what threatens the good manufacturing practices.

The deficiency in management and organization and lack of experience in the exercise of business roles, produces problems for the award and acceptance of roles. This is consistent with reports of PROINDER (2004) where the importance of trade-related organization, because a solid organization and a proper organizational structure, are the basis for achieving enterprise development is presented.

From this analysis of the situation, we inferred that its productive scenario is similar to that found in Peru in the late '80s, which offers the possibility of using the reported experiences to optimize the learning process necessary to achieve development local (Ureta Vaquero, 2007).

We observed farmers, transform their own knowledge and responsibility through their own participation, during the learning process. This was reflected in commitment in the activities, their continued contributions and concrete actions. Furthermore, the identification of constraints for each activity was facilitated by this process, whereby overcome these obstacles was transformed into a goal. This was also observed by Rogoff (1997) in his analysis of the three planes of sociocultural activity.

### ***3.2.1. Initial Situation***

Quebrada producers under analysis constitute an Aboriginal community with the possibility of access to subsidies that allowed them to acquire computer, camcorder and other properties. They have poor facilities for cheese making; the members of the community are the main producers of the fresh products produced and it is possible to buy raw materials to other local producers to increase production. We could verified that goat's milking is produced with the primary aim of making cheese for home consumption and the surpluses are sold without maturation and without packaging or protective cover, exposed on tables, on the edge of the road for the casual consumer. This

artisan cheese is made in rural establishments without adequate infrastructure, according to rudimentary practices transmitted generationally, with heterogeneous results in terms of organoleptic quality, mainly in the concentration and distribution of salt. Producers used raw milk (unpasteurized) with different times since the milking. The limited technology incorporated in the value chain of goat milk is mainly due to the low level of each producer individually considered, which is reflected in a low profitability of their global activity. Another influential variable is seasonality: between the months of April to September are not obtained volumes of milk that allow cheese making.

In the Quebrada, the development of informal economies of whole goat production process, from extraction of milk until cheese making and selling, is common in almost every family in the area. The management of herds with little treatment, manual milking and artisanal production, lack of technical and sanitary control, result in a product without adequate quality for markets outside the region, and even local, if they require sanitary conditions of the case. The deficiency in the marketing and the lack of training both in process as in management, are some of the disadvantages faced by producers. The future of a venture a higher scale, is subject to production levels, working capital, marketing, as well as pays tributes and formal market requirements. The development of agribusiness in subsistence contexts is possible if human capital accompanies the process and if it is socially contents in global vision. We can say that communities begin to walk the path to the RIA when they begin to combine a number of factors that relate to the characteristics of the community, related to the access to production factors provided by the context, the market and to the possibilities of technical support through the process of transformation of the community, in accordance with Boucher (2000), who compared the activation processes in 4 concentrations of small rural enterprises, milk processing. He Found that the main product derived from goat's milk is cheese, developed by individual actors in general, which does not guarantee its presence in the market.

### ***3.2.2. Intervention***

Intervention strategies found to achieve efficient linkage with the community can be summarized in a few assumptions:

Be respectful of their culture and interests: Any intervention began provided valuing their knowledge, their ways of thinking and feeling and ways of producing. This allowed identifying the procedures which need reformulation, which were related to good manufacturing

practices, sanitation and hygiene procedures and management strategies. Alternatives were proposed work, including the addition of new procedures and/or appropriate technology to its possibilities.

Estimate community interests in a process of co-construction of a common project, in a process of dialogue.

Find a language that is simple and convenient.

Search of work dynamics that support integration.

Respect their time without accelerating the processes of change.

### ***3.2.3. Construction of Vision***

The residents of the Quebrada are projected as recognized producer of differentiated products, of recognized quality, keeping their traditions and customs in the elaboration, pretending that this also be valued by the consumers.

### ***3.2.4. Development of Entrepreneurship***

Regarding entrepreneurship skills and access to productive factors, found that manpower incorporated into the process is represented mainly by the women of the family and the men are what primarily engaged in agricultural production that can be used as raw material for agro-industrial transformation processes.

It is considered an appropriate level of entrepreneurship development when achieved, at least, the realization of the imaginary and the obtaining of different production batches, marketing them, bearing in mind that to achieve this goal it was necessary to develop strategies for the supplying and organization of production, according to their capabilities. Accordingly, the ability of producers to entrepreneurship was conditional on the social and technical feasibility of carrying out the necessary actions. In this way, the first step was to determine if the community was truly interested in change their primary products to generate added value, what was verified in the community. The second step was to detect if the producers had at their disposal the necessary production factors: raw materials, capital and manpower and in case of not having, if it was possible to develop.

Throughout the research process and according to the responses and reflections of the goat farmers, the level of commitment they assumed was a determining factor in their ability to apprehension and openness to new approaches and ultimately in their response to actions associated with the planning and strategies applied to their production and marketing activities.



After six years of work and intervention in rural Aboriginal communities and evaluating the experience, have been collected lessons that is relevant to share. The heterogeneity of the organizations and community groups, allowed to identify relevant issues and obstacles that cannot be overcome by the community per se, but require technical support and management to make clarifying complex problem situations and define actions that enable them to meet their production targets. These difficulties seem more difficult to overcome by the lack of successful business models in the area.

Producers having succeeded in building his vision for the development and received technical inputs and specific training were able to identify and reflect on their problems productively and contrasted their situation with their expectations. This led them to demand specific information and analysis of development alternatives.

It was possible to generate a strategy of interaction considered as a methodology of mediation-intervention, which could replicate in different groups. The systematization is done consistent with the results published by Chavez-Tafur (2006). This methodology originated or was built sequentially from the reactions and questions of producers. It is synthesized through the following stages:

- 1) Elaboration of participatory diagnosis: survey of situation initial of group, identifying strengths and weaknesses to face a productive enterprise. They are valued traditional practices and culture of the peasants, through the implementation of strategies that give them a major role.
- 2) Building the vision: seeks to visualize the shared imaginary of rural group, to formalize in a project.
- 3) Awareness and training in good manufacturing practices: in regard to food enterprises, this stage plays a crucial role. He stressed the need to control the cleanliness and order as fundamental bases of a good working environment and product safety.
- 4) Productive processes - Review of the traditional process and identification of improvements: Implementation of documentation or records that endorse the assurance and quality control of product.
- 5) Cost analysis and price formation: identification of the costs of the factors involved in the production process for determining the price of the product. Consumer market analysis, determination of the potential demand and purchasing power of customers.

- 6) Organizational structure and assignment of roles: seeks the distribution of activities within and outside the organization as well as the form of profit sharing. We analyze the relationship between personal skills and roles that need to play within the enterprise, considering the skills demanded by each role.
- 7) Training and application of tools of marketing/management: as a first step should be to identify the legal requirements for operation of the establishment and marketing of product. Secondly, the product presentation and product positioning in the market. To introduce a new product on the market, is necessary to enable manufacturing facility by the competent authority as well as enable the product. To do this, must be met the regulations of *Código Alimentario Argentino*, which establishes nutritional labeling added to the product label.
- 8) Strategic planning for business: analyzed the 5 forces of Porter: suppliers, customers, competitors, those who perform a similar product and the productive sector itself, to develop strategies which give the product a competitive advantage, bearing in mind the previous stages that make to value chain.

### **3.2.5. Critical Points**

It was found that the main obstacles to the emergence of enterprise as a profitable activity are: 1) organizational deficiencies and 2) the idiosyncrasies of the peasant producer which limits their corporate vision and particularly in regard to marketing.

We identified the lack of implementation of measures to ensure the hygienic-sanitary quality of the product, resulting in insufficient development and use of farming/agribusiness as an alternative to generate income and improve the living conditions of local producers. Worked with producers on the need to take aware of the responsibility that this activity involves about health of consumers, because the goat cheese is sold in fresh Jujuy, namely without maturation that ensures a decrease in pH and  $a_w$  that provides greater security on the microbiological quality of cheese. This is one of the aspects most difficult to address, because if it is accepted the connotation it has on health, producers are reluctant to include the pasteurization as a fundamental operation. This resistance is based on the idea that pasteurization causes changes in the organoleptic characteristics of cheeses that these people are accustomed to consume. While this is true, the change to which they refer is associated with effects produced by undesirable microorganisms (coliforms), so it is necessary intense work in order to reverse these concepts, together with

the selection or development of an appropriate ferment obtained from microbiota of goat milk, to allow cheese making high-quality hygienic, with the organoleptic characteristics expected. There have been no other systematic studies to date, to know the nutritional quality of this goat milk and their derived products. Moreover, the *Código Alimentario Argentino* does not set standards for the hygienic-sanitary quality of goat milk, which makes it difficult to introduce this topic among small producers, reason why this work is a contribution to assist their inclusion in national regulations.

### ***3.2.6. Sustainability of the Project***

It was found that the activity takes place within a framework of subsistence. The main advantages are related to the possibility of having the support of different institutions and their own desire to improve.

It was observed that the value chain, at some point of the primary activities is truncated, sometimes by physical factors, in which raw material availability is not always made concrete (which makes that you cannot carry on a continuous production process) and other by human factors.

Considering that sustainability is possible when the sale of products enables the replacement of raw materials and cover costs as to generate an inventory to launch the business and that the circuit is closed when, at a level appropriate of entrepreneurship, sustainability is added, then we can say that the peasant producers of the Quebrada were able to achieve an adequate level of entrepreneurship development, but not the sustainability of their enterprises.

### ***3.2.7. Organization***

We found that the community has a strong leader who influences the people involved in value chain of goat milk. The assigned roles for production and commercialization are not yet put in practice.

### ***3.2.8. Strengths, Weaknesses, Opportunities and Threats to Development***

We could identify the following problems and solutions, with the producers together.

The weakness (problems), were:

- Low production, making it difficult to comply with the assumptions of continuity and quantity on offer.
- Little development of human capital and social capital.

- Low profitability, due to inadequate pricing and inadequate analysis of the costs
- Lower local commercialization and inexperience on the entry into new markets
- Little opportunities of access to capital
- Lack of quantitative data record which affects the capacity of sector planning and coordination among potential members of the value chain.
- Lack of pasteurization of milk
- Sales of cheese without packaging.
- Difficulty of incorporating the business vision.

The solutions found by the producers were:

- Identification of new products development.
- Search of markets to insert the products
- Include a total cost analysis
- Capacitating in elaboration process and good manufacturing practices incorporation, as so incorporation of sanitary procedures and technology acquisition.
- Organization of tasks: assignment and allocation of roles among members of the community.

We see that the problems as solutions detected by producers are similar to other Andean regions, in coincidence with Boucher (2000).

The main threat is related to the fact that most producers have government plans for economic assistance, creating a "competition" with rural productive activity. Moreover, the nature of the Jujuy market, sensitive to the price of substitute product "cow cheese", is another threat to small-scale activity, for what it is difficult to set a standard of achievement in terms of production and prices, to achieve profitability; also the food control authority is intensifying controls in the area, making it impossible to maintain the activity without the incorporation of changes.

As opportunity, we can highlight the fact that the Quebrada de Humahuaca has been declared by UNESCO in 2003 Cultural Heritage of Humanity, which transformed it into an attractive market for national and international tourists, mostly Europeans. To this must be added that in recent years was changing food consumption patterns, especially, urban consumers in more developed countries, where the rurality, the rescue of typical products,

the naturalness with their respective traditional process, form the new imaginary on food consumption (Von Hesse, 1994; Bernat, 1996). The ability to interact with the producers of the Valleys (more developed area, with better infrastructure and higher production) provide an opportunity to access markets, taking advantage of having higher production volumes. For it is necessary to incorporate actions to unify and maintain quality, incorporating management tools with cluster strategies.

Indigenous peoples of the Andean countries have made little progress in economic and social development during the last decades, and certainly are social groups that have the highest levels of poverty, less education, higher incidence of disease, exclusion and discrimination. Rural poverty is manifested through marginalization, discrimination against rural women, the lack of investment in human capital, unequal land tenure, increasing levels of food insecurity, rural migration, lost identities and this, added to the growing lack of public services, technical assistance, training, business organization, credit and research, among others, determines the need to support communities in their development process, with continuous activities, sustained over time, because delivery by the State, in different plans, of financial and physical resources is not enough. That is why, that role played the development and maintenance of the RAI in the region, is essential to accompany the process of territorial development, which necessarily encompasses both the economic and social issues, those that must be solved to give base and support to any productive venture.

## CONCLUSION

The goat race, the lactation cycle and some factors like availability of food (and / or lack of supplementation) and the usual practices of animal management, influence the quality and admissibility of milk destined to process.

The lactation cycle of Valleys is about 10 month, distributed in four periods, meanwhile in Quebrada, the cycle includes 4 month in two periods.

The milk production level in Valleys is higher significantly to Quebrada.

The higher nutrient contents are obtained in December, at the beginning of the raining season, in both zones.

The main differences in composition between zones are obtained in protein and fat.

The milk composition is strongly related to seasonal changes and food availability for goats and therefore to the nutritional state of goats.

The physicochemical composition results of analyzed milk showed aptitude to cheese manufacturing, featuring high cheese yields.

The cheeses showed adequate quality to commercialization after implementing a stage of pasteurization.

The compositions of the cheeses present significant differences between races and vary with production zone.

There was a great variability in composition, both in milk as in cheese, compared with other zones of goat milk production, influenced by animal feeding, geographic location and climatic characteristics.

The real yield of cheese elaboration was 15.27% and the calculated with Emmons equation was 15.68% with 2.7% error.

The agro-industries development is possible, in subsistence context where resources can be available circumstantially, if human resources are prepared to accompany the process. This implicates a slow process that the producers need experiment to ensure, enhance and consolidate their organization.

The problematic of goat sector in Valley and Quebrada are focuses mainly in under development, the informality of commercialization logistic and deficiencies in milk and cheese microbial quality due lack in application of good manufacture practices. The low productivity due low yield of milking and seasonality of raw material influences in the problem.

The application of the strategies founded in this study could sustain the comprehension and the identification of obstacles to improve the producer's organization. The interaction between the producers of Valley and Quebrada for commercialization, besides the recollection and systematization of data that would collaborate in decisions, producers could access financial support to invest in infrastructure and new technology. This would allow them to introduce the local cheese production in markets of delikatessen or exotic products, using a comparative and competitive advantage.

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*Chapter 2*

# **PRODUCTION OF GOAT’S MILK AND FATTY ACIDS PROFILE: A NEW PERSPECTIVE IN HUMAN DIET**

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## **ABSTRACT**

World production of goat’s milk is estimated at 15.5 million tons annually, as it is considered a highly prized product it is easily produced on a small areas, also a tool to increase the sustainable profit ability of the farmer. Because of the higher digestibility and hypoallergenic properties of goat’s milk, this is mainly consumed by the elderly, sick as well as infants. It is estimated that symptoms of cow milk allergy occur between 2.5% to 7% of infants, and research suggests that one third of allergic new-born Infants to cow’s milk are tolerant to goat’s milk, however often there is confusion between intolerance and milk allergy, mainly caused by alpha-s-1-casein, abundant in cow’s milk. Goat’s milk has a guaranteed space in the market, due of it is high biological value and low allergenicity, when compared to cow’s milk and soya milk. However, there are obstacles to expansion the dairy goat market largely because of: instability in the product offering; cultural resistance of consumption, with the “goat being the cow of the poor people” and low acceptance due

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to the typical flavor or poor flock management. The milk and dairy products provide a large portion of saturated fat consumed and some fatty acids that are essential to human nutrition. In the recent past, milk and dairy products have become unpopular between nutritionists, but the bad reputation of saturated fatty acids, should not be widespread, since today it is known that stearic acid (18:0) has no the atherogenic effects. The essentiality of certain fatty acids has been established by the several researches due to the inability of animals and humans in synthesizing them, and its deficiency causes disorders of growth, changes in the skin, several behavioral disorders, immunological and neurological changes. Comparative studies used as references by medical associations and health institutes for the dietary reference intakes (DRIs), compares the western diets with Greek diet and the Paleolithic period, through simulations based on modern-day hunter-gatherer populations, suggests a ratio of omega 6:3 of 1-2:1, consumption much lower in saturated fatty acids, total fat and almost no of trans fatty acids, except the CLA, being more balanced and healthier than does today's diet. This is evidenced by lowest rates of cardiovascular disease, cancer, infant mortality and highest life expectancy in the Greek Islands, where the diet consists largely by Mediterranean plants and moderate portions of yogurt and cheese from goats and sheep reared on pasture. However, care should be taken in causal studies, since the origin of carcinogenesis and cardiovascular diseases is multifactorial. Thus, there is a challenge to improve the ratio of hypocholesterolemic and hypercholesterolemic fatty acids and decrease the atherogenic index to human health, which in the next few years can become a tool in the promotion of production systems of dairy goats based on pasture and supplementation.

**Keywords:** Boer, CLA, forage, omega-3, PUFA, goat milk

## 1. INTRODUCTION

The rearing of goats, as all economic activity, aims to increase the efficiency and improvement in productivity rates, as well as a reduction in production costs (Ferreira, 2002). The purpose of this review is explain the changes on world production of goats, the specific characteristics of physico-chemical properties of goat in comparison with those dairy species, with emphasis on lipid profile of goat's milk and what its importance on human health as a functional food.

## 2. WORLD GOATSTOCKS

### a) Goat Stocks in Developed and Developing Countries

An approximate increase of 233% in goat stocks occurred worldwide from the 1960's to 2009 (FAO, 2011; FAO, 2006), reaching a level of 879.7 million head in 2009, of which approximately 96% were in developing countries, as can be seen in Figure 1, adapted from FAO (2006). World goat stocks have been increasing since 1960-65 by about 3% each year (FAO, 2011).

The highest number of goats is observed in Asia, followed by Africa, representing approximately 59.7% and 33.8%, totaling 93.5% of the world population, respectively.

The lowest number of goats is found in Oceania, accounting for 0.1% of the total world population (Aziz, 2010). The country with the highest number of goats in the world is China, followed by India, Pakistan and Bangla-desh; together these countries constitute approximately 45% of the world total.

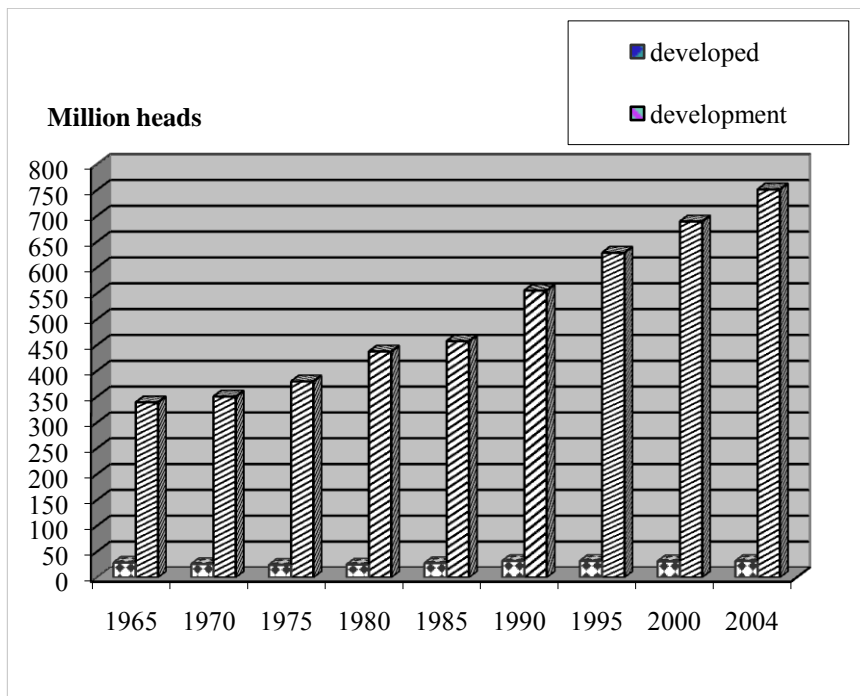


Figure 1. Goat population (million heads) in developed and developing countries 1965-2004 ((FAOSTAT, 2006).

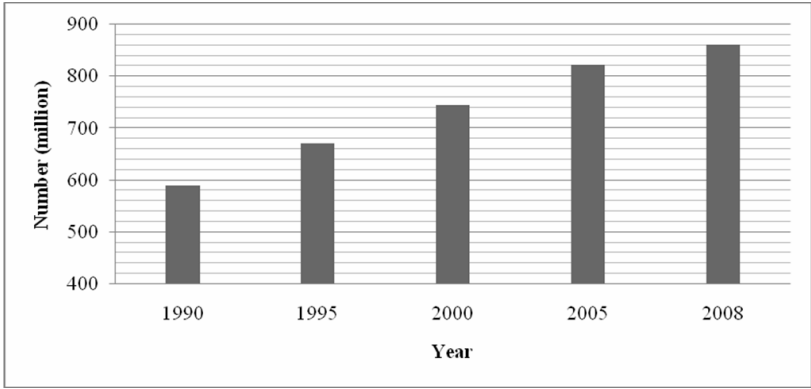


Figure 2. Worldwide goat population 1990 to 2008 (FAOSTAT, 2011), adapted from Aziz, 2010.

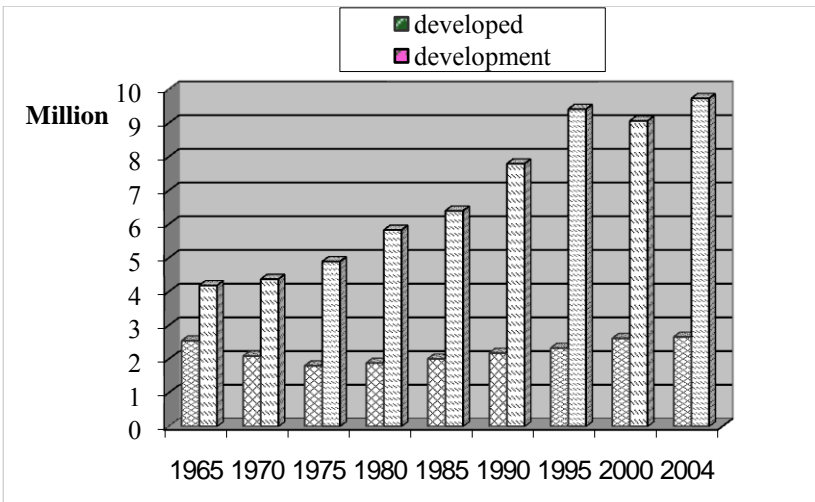


Figure 3. Goat Milk production (millions of tonnes) in developed and developing countries from 1965 to 2004.

## b) Goat Milk Production in Developed and Developing Countries

The official production of goat's milk shows a different characteristic when compared to the worldwide distribution of herds, with 78.6% and 28.4%



for developing and developed countries, respectively (Figure 3), so a relative concentration of milk production in developed countries has occurred, with just 4% of livestock's goats producing 28.4% of worldwide goat's milk.

However, the new world economic order tends to change this fact. World production of goat's milk is estimated at 15.5 million tons annually, as it is considered a highly prized product and is easily produced in small areas, as well as being tool to increase the sustainable profitability of the farmer, and it was distributed mainly in Asia, followed by Mediterranean European and part of Africa (Figure 4).

The highest goat milk production is observed in India, Bangladesh and Sudan (FAO, 2011). The European countries such as France, Greece and Spain have a strong impact on world exports of cheese and fresh milk of goats. Haenlein (2001) estimates that at least 10 countries depend on goats and sheep to supply between 30 and 76% of total milk consumed.

However, less than 5% of the total milk produced by goats is marketed (Aziz, 2010). Seasonal breeding and the resulting annual fluctuations in goat milk supply have made development of new markets difficult and have dampened the importance of milk yield as a breeding goal (Haenlein, 2001).

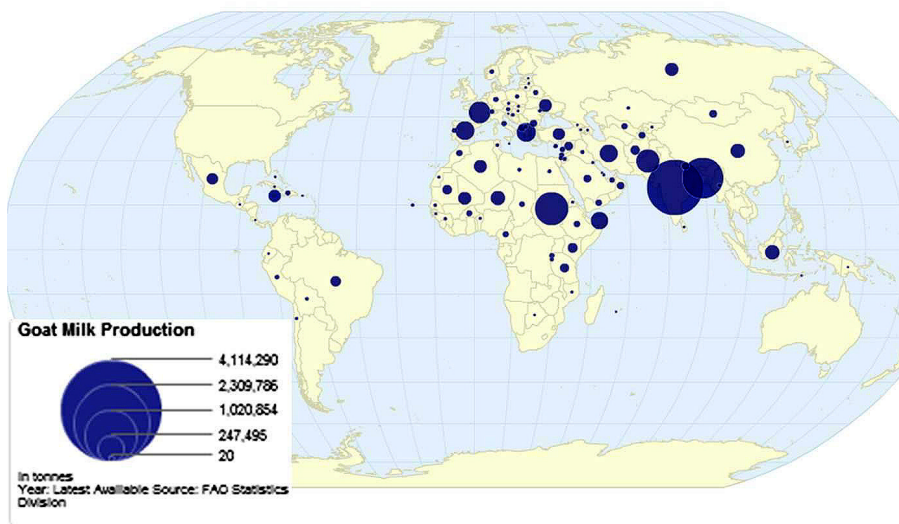
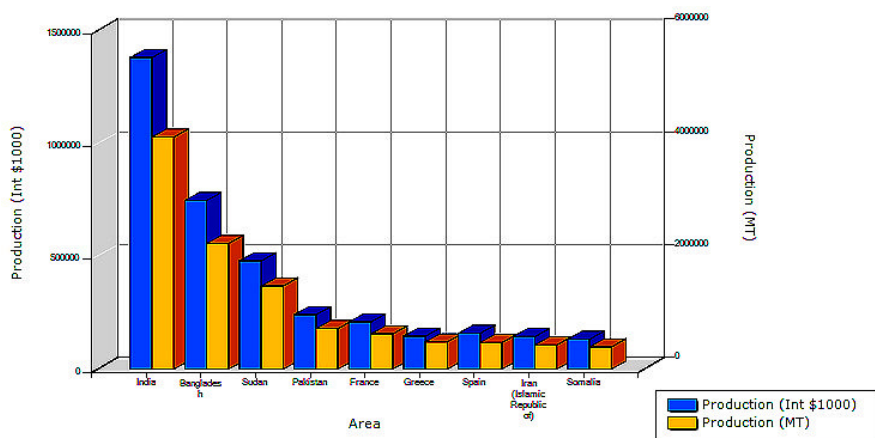


Figure 4. Current worldwide distribution of goat milk production (tonnes) in 2009. Source: FAO, 2011.



Rank	Area	Production (Int \$1000)	Flag	Production (MT)
1	India	1380673	*	4114290
2	Bangladesh	747303	*	2226900
3	Sudan	480131	*	1475000
4	Pakistan	241282	*	719000
5	France	208885	*	623460
6	Greece	146178	*	484000
7	Spain	158729	*	473000
8	Iran (Islamic Republic of)	143991	*	429082
9	Somalia	132511	*	394873
10	Niger	92650	*	276091
11	Indonesia	92620	*	276000
12	China	87147	*	272443
13	Russian Federation	78819	*	234875
14	Mali	75108	*	223818
15	Algeria	74074	*	220736
16	Ukraine	7718	*	218200
17	Jamaica	68241	*	203353
18	Turkey	64501	*	192210
19	Mexico	56947	*	169698
20	Brazil	48245	*	143768

Figure 5. Top twenty global goat milk producers in 2009, production in MT of milk and in 1000 dollars.

### c) Goat Meat Production in Developed and Developing Countries

It should be noted that although the production of meat generally corresponds to the distribution of the world's livestock population, with 95.5% of meat production concentrated in developing countries and 4.5% concentrated in developed countries (Figure 6).

The highest amount of goat meat is produced in China, India, Nigeria, Pakistan and Bangladesh, all of which produce above 200 million tons of goat meat (FAO, 2011). Meat production has gained expression with a genetic improvement through the introduction of Boer breed, industrially or absorbed crossbred with the existing flock.

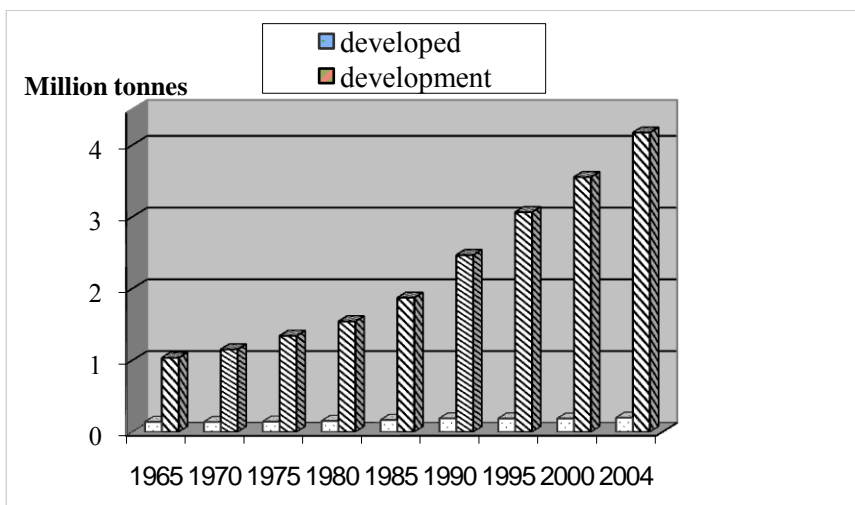


Figure 6. Meat production (million tons) in developed and developing countries from 1965 to 2004.

These animals from South Africa are characterized by their precociousness, performance excellent in weight gain and uniformed carcass. Prices range from 3.5 to 6.7 dollars per kg body weight for kids on Brazil.

In the dual purpose system provide milk and meat production, the use of Boer breed to crossbreeding in traditional dairy goat farming systems is widespread especially in developing countries such as China, Brazil and South Africa, however the same phenomenon also exists in many developed countries, such as the U.S. and Australia. In many countries, especially those

developing ones, it is used for production of crossbreed females to milk and crossbreed males to meat production, despite a lack of research proving the feasibility of this alternative to maximize the gains from dual purpose system. This phenomenon of absorbent or terminal crossbreeding has changed the profile of the dairy goat farms in some traditional regions of southern and southeastern Brazil, which currently produce meat goats, as a result of crossbreeding with absorbent Boer sire about the original dairy herd. The use of these specialized animals and products derived from crossbreeds requires proper nutritional plan use in their raising (Vieira et al, 2005), and in these cases, the supplementary feeds may be employed in order to minimize the effects of seasonality on the feed availability to keep average gain rates zoo-technical appropriate. (Joemat et al., 2004).

The goat meat and its products show some benefits in relation to beef or other meat, has considerable nutritional value of iron and protein and lower in fat than chicken, beef, swine and sheep. To take advantage of goat meat is needed use an attractive packaging that enhances the natural characteristics of the product, through the use of spices and cuts themselves, which certainly contribute to consolidating the productive chain, coupled with a good marketing through advertising, television or internet, festivals and promotion of culinary tastings (Osmari, 2009).

#### **d) History and Goat Production in Brazil**

In this context, Brazil stands out with a goats population of 9.31 million (IBGE, 2010), the 17th largest worldwide flock, occupying the 27th and 20th position in world production of meat and milk goats, respectively. Although about 90% of the herd is concentrated in the Northeast (IBGE, 2010), goat farming has also aroused interest in other regions, notably in the South and Southeast, which is mainly focused on the market for milk and dairy products (Zacharias, 2001). Recently, the market for goat meat is also growing in these regions (IBGE - Municipal Livestock Survey, 2010), that is related to the organization of producers associations and effectiveness of regional public policies over the last years. The production of goat milk in Brazil was estimated at 143,768 million tons in 2009 (FAO, 2011), being a source of nutrition and income in the poorest regions such as the northeastern backlands.

Commercial goat milk production in Brazil has increased since the 1970's, mainly because of to the need for alternatives to high-quality products and profitability, occurring initially in the Southeast and later followed by the

South. In 1994, European countries launched fluid milk through the process of ultra-high temperature UHT, changing the world stage, according to Zacharias (2001), because to date, almost all goat milk produced in Europe was for the manufacture of fine cheeses. The consumer has adapted quickly to new presentation, according to market research in 2000 and mentioned by the same author, in which 62% of type C milk sales in 1995 were reduced to 36% in 1998, according to storage and the possibility of microbiological quality higher. At the same time, there was also a significant increase in official data of goat's milk production in Brazil with the absence of a comparably organized infrastructure, promotion system and market expansion, leading to a drop in prices and subsequent decline of herd number, especially in South and Southeast regions, milk production traditionally (Table 1).

**Table 1. Brazilian goat herd - effective by region**

(Thousand Heads)

Regions	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
North	108	112	123	135	139	137	140	140	155	155	167	176	177	164
Northeast	7,418	7,596	8,033	8,741	8,909	8,790	8,906	8,906	9,543	9,614	8,634	8,521	8,303	8,459
Southeast	193	199	204	204	211	211	226	226	252	263	253	226	232	233
South	174	179	182	182	187	195	206	206	243	252	280	318	336	343
Midwest	75	77	81	85	92	96	104	104	114	117	116	113	116	113
Brazil	7,968	8,164	8,623	9,347	9,537	9,429	9,582	9,582	10,307	10,401	9,450	9,355	9,163	9,312

Source: IBGE - Municipal Livestock Survey-PPM ([www.ibge.gov.br](http://www.ibge.gov.br)).

However, the Brazilian goat herd began to grow again in the late 90's until 2004, at a slower pace, as can be seen in the data between 1997 and 2004 of table 1, when the dairy goat industry began to be better organized. Currently, a slight decline and herd stagnation is occurring in the northeast, however, the introduction of the Boer has grown in South and Southeast regions, even in dairy herds, whereas the Boer/dairy 50% crossbred have some dairy potential (Osmari et al., 2009) and price of a crossbred kid is greater than the value of a purebred dairy kid, similar to what happens in some regions U.S. It is worth noting that the main form of goat milk consumption in Brazil is informal,

therefore is not detected by official agencies, besides the fact that many producers are replacing the dairy herd by new exotic meat breeds such as Boer.

Therefore, most developing countries need research, extension service, and support through public policy to improve apparent productivity of goats and sheep. (Haenlein, 2001). The author point out two major research needs transcend all improvement and development topics:

- Identify better and promote the marketing of the unique values of dairy products from goat and sheep's milk, so to better justify their existence and higher prices for their products;
- Improve conditions of family livelihood and better net income levels of small ruminant farmers to achieve keeping the farmer and his family content in this enterprise and reduce off-farm migration.

### **3. PROPERTIES OF GOAT MILK**

#### **a) Goat's Physicochemical Analysis and Composition**

Goat's milk can add value to the producer because it is used primarily by the elderly, sick and children, for its high digestibility. In the past milk consumption was encouraged, but recently it has been discouraged because of concerns about its fat content. Ness et al (2001) found no evidence that milk consumption was associated with increased risk of coronary death or death from all causes, and verified that milk drinkers were healthier in several respects. The researchers also state that a residual confounding may explain the failure to observe increased mortality in milk drinkers.

Information on composition and physicochemical characteristics of goat's milk is essential for successful development of dairy goat industries, goat producers, as well as for the marketing the products.

South Africa and the United States pioneered the use of goat milk, including evaporated or powdered, for children. In the 70's, during two years, 1975 and 1976, evaporated goat's milk was used in more than 100 000 children in South Africa, and no cases of anemia was reported in relation to goat's milk (Maree, 1985) proving its effective.

Compositions of goat, sheep, cow and human milks are different (Park et al., 2007; Parkash and Jenness, 1968 ;Jenness, 1980), and vary with diet,

breed, individuals, parity, season, feeding, management, environmental conditions, locality, stage of lactation, and health status of the udder ness in child nutrition.

Proteins are composed of amino acids with a chain of physical and chemical properties very different, which explains the different effects of different proteins in human digestion and metabolism (Haenlein, 2002). Protein contents vary widely within species, and are influenced by breed, stage of lactation, feeding, climate, parity, season, and udder health status. Goat and sheep milk contains about 0.7–1.0% and 0.4–0.8% N, respectively, which is distributed in fractions, whose importance varies in terms of dairy technology and human nutrition (Park et al, 2007). In studies with rats, which had malabsorption syndromes, it was found that goat milk improved the intestinal absorption of copper, which was attributed to the higher contents of cysteine (derived from cystine) in goat milk (83 mg/100 g) than in cow milk (28 mg/100 g) (Barrionuevo et al., 2002). Overall, the adult daily dietary nutrient recommendations for essential amino acids would be met equally or exceeded by a 0.5 l goat milk consumption compared to cow milk (Haelein, 2004).

Since a change in only one amino acid can make a big difference in how this protein acts and reacts, and their influence on uniformity in the processing of cheese (Imafidon et al. 1991). Maree (1985) reports in his review, that Hofman (1958), showed by electrophoresis that the distribution of various components of the casein from bovine milk and goat's milk are completely different. The casein in cow's milk consists of 55% alpha-casein, 30% of beta-casein and 15% of kappa-casein, while goat's milk contains 19% alpha 1, alpha 2 21% and 60% beta-casein. One of the relevant works which contributed immensely to our knowledge of goat's milk on the polymorphism of molecular structure of casein was carried out in France by Duman et al (1975). It was for the first time possible to demonstrate the molecular structure of bovine milk casein.

The molecular structure of goat's milk was done immediately afterward proving beyond doubt that goat's milk casein differs widely in basic chemical structure from that of cow's milk. The beta-casein in goat's milk is more soluble on cooling than the homolog in cow's milk. In a study of digestion of kappa-casein, 27 differences in amino acid sequence between caseino-macropeptides casein milk cow and goat were found (Mercier et al., 1976). The low casein content and other characteristics such as alphaS1-casein proportions and micellar size are believed to be responsible for the weak texture of goat's milk yogurt. The casein content of milk has a significant influence on rheological properties of the rennet gel, its setting speed and its maximum

firmness (Park et al, 2007). Rennetingtime for goat milk is shorter than for cow milk, and the weak consistency of the gel explains mediocre cheese suitability of goat milk.

Related studies (Zeng et al. 2007; Damián et al. 2008) about the genetic polymorphism of alphaS1-casein in goat milk and observed that goat milk with different levels of alphaS1-casein resulted in significantly different in cheeses yield. Damián et al. (2008) also reported differences between Anglo-Nubian and Saanen breeds, and between types of casein to the cheese yield. Their discoveries about the influence of protein type could explain the low correlation of protein content in present goat milk with yield predicted of goat cheeses, since casein is the major component of milk protein (Osmari et al, 2010).

Besides the main caseins (alphas1-CN, alphas2-CN, beta-CN and kappa-CN), the milk protein have other fractions of protein, as casein macro-peptides (CMP), which are the soluble C-terminal derivatives from the action of chymosin on kappa-casein during the milk clotting process of cheese making, which have been identified as a good source of antithrombotic peptides. The goat milk protein also has bioactive angiotensin converting enzyme (ACE) inhibitory peptides that control microbial infections and antihypertensive peptides (Park et al, 2007).

The average amino acid composition of goat and cow's milk, as published in the official USDA tables, shows higher levels of six of the 10 essential amino acids in goat milk: threonine, isoleucine, lysine, cystine, tyrosine and valine (Haenlein, 2004., Posati and Orr, 1976). Several authors (Jenness, 1980; Ribeiro, 1998, Ribeiro et al, 1997) have reinforced the low levels in goat milk compared to bovine milk for alphaS1-casein, beta-carotene, and agglu-tinin. Several researchers found differences for all other fractions of the protein between goat's milk and cow's milk, besides lactalbumin. This would be the likely reason why an infant to a product intolerant of cow's milk and often tolerate goat milk.

Ribeiro (1998) relates that the agglutinin, a substance present in cow's milk that binds the fat particles, forms a curd which in turn is more difficult to digest.

Regarding the main source of carbohydrate in milk fat, lactose content of goat milk is about 0.2–0.5% less than that of cow milk (Posati and Orr, 1976; Park et al, 2007).



**Table 2. The composition of goat's milk compared to human's milk and cow's milk (values per 100 ml)**

Constituents	<i>Human</i>	<i>Cow</i>	<i>Goat</i>
Protein (g)	1.2	3.3	3.3
Casein (g)	0.4	2.8	2.5
Lactalbumin (g)	0.3	0.4	0.4
Fat (g)	3.8	3.7	4.1
Lactose (g)	7.0	4.8	4.7
Caloric Value(Kcal)	71	69	76
Mineral matter(g)	0.21	0.72	0.77
Calcium (mg)	33	125	130
Phosphorus (mg)	43	103	159
Magnesium (mg)	4	12	16
Potassium (mg)	55	138	181
Sodium (mg)	15	58	41
Iron (mg)	0.15	0.10	0.05
Copper(mg)	0.04	0.03	0.04
Iodine(mg)	0.007	0.021	-
Manganese (mg)	0.07	2	8
Zinc (mg)	0.53	0.38	-
Vitamins:			
Vitamin A (I.U.)	160	158	120
Vitamin D (I.U.)	1.4	2.0	2.3
Thiamine (mg)	0.017	0.04	0.05
Riboflavin (mg)	0.04	0.18	0.12
Nicotinic acid (mg)	0.17	0.08	0.20
Panthenic acid (mg)	0.20	0.35	
Vitamin B6 (mg)	0.001	0.035	
Folic acid (magnetocardiogram)	0.2	2.0	0.2
Biotin (magnetocardiogram)	0.4	2.0	1.5
Vitamin B 12 (magnetocardiogram)	0.03	0.50	0.02
Vitamin C (mg)	4.0	2.0	2.0

Source: Maree (1985).

Generally, changes in goat and sheep milk compositions occur by seasons, because towards the end of the lactation, the fat, protein, solids and mineral contents increase, while the lactose content decreases, although health pro-

blems of the mammary gland can reduce the total solids in general. Lactose is a milk sugar, synthesized from glucose in the mammary gland with the required active participation of the milk protein alpha-lactalbumin (Park et al, 2007).

With respect to lipids, with respect to lipids, are the most important components of milk in terms of cost, nutrition, and physical and sensory characteristics that they impart to dairy products. Triacylglycerols (TAG) constitute the biggest group (nearly 98%), including a large number of esterified fatty acids, with composition very complex.

Along with TAG, the lipid composition of ewe and goat milk presents other simple lipids (diacylglycerols, monoacylglycerols, cholesterol esters), complex lipids (phospholipids) and liposoluble compounds (sterols, cholesterol esters, hydrocarbons) (Park, 2007). The average fat globule size is smallest in goat's milk, what is advantageous for digestibility and a more efficient lipid metabolism compared with cow's milk fat. With the exception of Mehaia (1995) which found the lowest globule size in goats, Park et al (2007) reported general average of research to fat globule sizes in this decreasing order: cow > goat > ewe.

The contents of citric acid, sodium, sulfur, zinc, ribonuclease, alkaline phosphatase, lipase, xanthine oxidase, pyridoxine, folate, vitamin B12, vitamin C, freezing point from cow's milk are lower than found for the goat's milk, while contents of calcium, potassium, magnesium, phosphorus, chlorine, manganese, vitamin A, vitamin D, niacin, choline, inositol, medium-chain fatty acids, fat globules of smaller diameters and somatic cells, are higher compared to bovine milk (Droke et al, 1993). Compared to cow's milk, there are contradictions in the literature for the levels of iron and zinc, and is considered higher in goat milk in Haenlein (2002), and lower by Maree (1985), the latter author made a compilation of data analyzed in 10 different countries represented in Table 1. Haenlein (2002) reported in his review lower values for the goat's milk for potassium, contrary to data presented by Maree (1985) and Droke et al (1993) for this mineral, however all the aforementioned authors found lower or equivalent levels of vitamin C than that found in bovine milk. Already Ribeiro (1998), in Brazil, also citing Jandal (1996) reported high levels of vitamin C and vitamin B12 for the goat's milk compared to cow's milk and even in relation to human milk, contradicting the data cited.

Haenlein (2002) comparing review about milk from sheep, goat and bovine found the lowest levels for copper, iron, zinc, manganese, calcium and magnesium in milk of Frisian cow breed, high content of minerals were found in the milk of Merino sheep, with the exception of manganese, where the

goat's milk Serrana-Andalusa breed was higher, while in the other two species it was intermediate, the sodium was higher in cow's milk, followed by sheep's milk and goat's milk, while potassium was higher in cow's milk, followed by goat's milk and sheep's milk.

The supply of two glasses (1/2 L) of milk or cheese or yogurt equivalent from goat's milk will provide up to 94% of recommended adult daily dietary allowances (RDA) of essential amino acids, 83% of calcium, 78% of riboflavin, and other minerals and vitamins to a lesser degree, while supplies from cow milk are up to 81, 74, and 89%, respectively, according to Haenlein (2001).

## **b) Goat's Milk as Food Hypoallergenic**

Treatment with goat milk resolved between 30 and 40% of the problem cases, and in one particular study 49 of 55 treated children benefited from treatment with goat milk. Allergy to cow's milk is considered a common disease, according to Haenlein (2004), which in its survey data, reported 2.5% prevalence in children during the first 3 years of life (Businco and Bellante, 1993), occurring in 12-30% of infants less than 3 months old in the study of Lothe et al. (1982), an overall frequency of 7-8% in Scandinavia (Host et al., 1988), reported in 3% of children under 2 years of life in Italy (Bevilacqua et al., 2000) and even rates as high as 20% in some areas (Nestle, 1987). Haenlein (2004) states that treatment with goat's milk solved 30 to 40% of cases in a particular study in which of 55 children, 49 benefited from treatment with goat's milk. Park (1993) affirmed that between 40 to 100% of patients allergic to cow milk proteins tolerate goat milk.

This data presented are in agreement with the general estimate of the symptoms of allergy to cow's milk which occur in 2.5 to 7% of infants, and with the suggestion that one third of allergic newborns to cow's milk can tolerate goat's milk (Maree, 1985; Zacharias, 2001). There is often confusion between lactose intolerance and milk allergy, mainly caused by alphaS1-casein, abundant in cow's milk and with antigenic structure distinct than the present in goat milk. Cow's milk allergy (CMA) is a common disease of infancy and childhood. Given this range of elements that promote allergenicity relatively low when compared to cow's milk and soy, among others, goat's milk has also secured its space due to its high biological value. Maree (1985) points out that the real prevalence of allergenicity to cow's milk remains unknown, because the estimated data of literature do not always consider that

some disturbances produced by milk or its components cannot be assigned to the same antigenicity, where other factors such as: specific toxins, physical properties, poor preparation, bacterial contamination or the possibility of antibiotic residues used by the producer of milk.

The role of the indiscriminate use of antibiotics by producers of milk may also be an allergic factor and has yet to be evaluated. Maree (1985) citing the research of Jester et al. (1959), reported that penicillin concentrations from 0.006 to 1.22 u/mL were found in 3.5% of 1170 samples of cow's milk examined, while in 24 samples other antibiotic agents were also detected.

Still occurring barriers to worldwide expansion of consumption of goat's milk, such as: in relation to the instability of supply of the product, lack of consumption habit of cultural order (goats are generally regarded as poor man's cow), lower acceptance due to typical flavor or poor hygiene of management. However, the trend of increasing scale dairy with a consequent decrease of the final price to the consumer together with public awareness of its nutraceutical value and slow but progressive modernization can corroborate to change this situation.

#### **4. FATTY ACID PROFILE OF GOAT MILK AND HUMAN DIET**

The milk and dairy products provide a large portion of saturated fat consumed by humans, about 25-35% of the overall saturated fat intake, and recently, have become the preferential target of several nutritionists (Chilliard and Ferlay, 2004), but should not be widespread, since today it is known that stearic acid (18:0) has no the atherogenic effects. Ruminant's milk fatty acids (FA) are linked to intrinsic (animal breed, genotype, lactation and pregnancy stages) or extrinsic (environmental) factors; whereas feeding and lactation stage effects are quantitatively more important than the cheese-making technology on modulation of milk fatty acids.

Goat milk exceeds cow milk in contents of fatty acids (FA) mono-unsaturated (MUFA), polyunsaturated fatty acids (PUFA), short (SCFA) and medium chain fatty acids (MCFA) especially in caproic (C6:0), caprylic (C8:0), capric (C10:0), which all are known to be beneficial for human health, especially for cardiovascular conditions.

Three of the SCFA have actually been named after goats, because of their predominance in goat milk, and are associated with the characteristic flavors

of cheeses and can also be used to detect admixtures of milk from different species. The biomedical superiority has great potential in justifying the uniqueness of goat milk in human nutrition and medicine (Babayan et al, 1981; Haenlein, 2004), but is not widely publicized. The poor reputation of saturated FA, however, should be re-evaluated, since Chilliard and Ferlay (2004) point out that stearic acid (C18:0) is not related to increased cholesterol or atherogenic effect because it is metabolized to oleic acid (C18:1), with levels of 6–11% of C18:0 and 18–23% of C18:1 being nutritionally desirable, while the allegedly atherogenic effect of certain trans monounsaturated fatty acids, such as vaccenic (trans11-18:1), a major trans isomer present in milk, has not been confirmed.

On the other hand, the interest of increasing the n-3/n-6 ratio of polyunsaturated fatty acids (PUFA) has been confirmed (Chilliard and Ferlay, 2004; Cordain et al, 2005; Simopoulos, 2008) as well as the properties of the acid conjugated linoleic acid (CLA) whose main isomer, rumenic acid (cis9,trans11-18:2), is related to the prevention of certain forms of cancer and obesity, besides other attributes of a functional food.

Furthermore, the consumers are more interested in products enriched with these substances of interest and are inclined to pay for them.

### **a) The Importance of the Omega-6/Omega-3 Fatty Acid Ratio**

Currently, several biochemical studies seek greater distinctions between the unsaturated fatty acids (UFA), because of identification of beneficial UFA with long-chains which are more related to the properties formerly attributed mainly to short-chain fatty acids. These UFA are grouped mainly in families known as omega, as reported Simopoulos (2008), divided into groups omega-9 (monounsaturated), omega-3 and omega-6 (polyunsaturated), and the set of conjugated linoleic acids (CLA) and the pool known as conjugated linoleic acids (CLA). Some essential fatty acids to human nutrition justified the interest of increasing the n-3/n-6 ratio in human diet. The essentiality of certain fatty acids has been established by the several essays of research due to the inability of animals and humans in synthesizing them, and its deficiency causes disorders of growth, changes in the skin, several behavioral disorders, immunological and neurological changes.

In the absence of essential fatty acids (omegas 3, 6 and 9), the metabolism of animals, including humans, introduces a double bond between carbon atoms of 9.10 stearic (C18:0) to form oleic acid, which in turn, through the

elimination of hydrogen and carbon chain increased, functions as a precursor for FA omega-9. The same happens with the linolenic acid, which acts as a precursor of other fatty acids from the manipulated group omega-3. The major LCFA manipulated in the diet of ruminants are CLA, omega-3 and omega-6.

Several sources of information suggest that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of ~1 whereas in Western diets the ratio is 15/1–16.7/1 (Simopoulos, 2008; Simopoulos, 2000).

Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Hughes-Fulford et al, 2005), whereas increased levels of omega-3 PUFA (a lower omega-6/omega-3 ratio), exert suppressive effects (Simopoulos, 2008).

Comparative studies used as references by medical associations and health institutes for the dietary reference intakes (DRIs), compares the western diets with Greek diet and the Paleolithic period (Figure 7), through simulations based on modern-day hunter-gatherer populations, suggests a ratio of omega-6/omega-3 of 1-2/1, consumption much lower in saturated fatty acids, total fat and almost no of trans fatty acids, except the CLA, being more balanced and healthier than does today's diet.

The study of the Lyon Diet Heart illustrated the significant impact of a diet lower in fat omega-6, with an impressive reduction in mortality from heart disease (Simopoulos, 1999). Before industrialization, no population has been exposed to the current high levels of dietary linoleic acid (Simopoulos, 2001). Today's current intake is a type of fat does not exist 150 years ago, such as cotton seed oil and margarine (Simopoulos, 2001; Chilliard and Ferlay, 2004; Simopoulos, 2008).

Gagliardi et al (2009) analyzed the relation 6/3 in fried potatoes, stuffed sweet cookies and salty crackers from a multinational chain of fast food all with allegation of 0% trans fatty content, all were purchased in commercial points on Brazil. They found 70/1, 90/1 and 90/1 ratios, respectively, well above the level recommended by FAO-WHO, much worse than the values in dairy products.

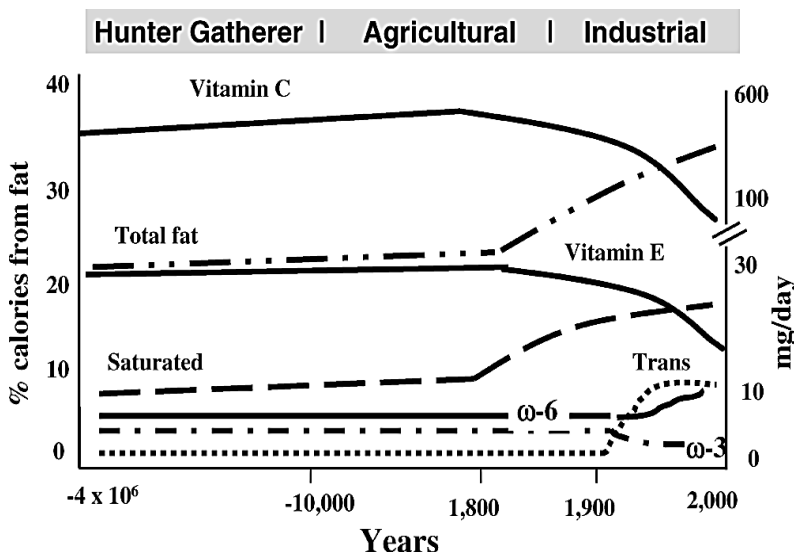


Figure 7. Hypothetical scheme of fat, fatty acid (n-6, n-3, trans and total) intake (as percentage of energy from fat) and intake of vitamins E and C (mg/d). Source: Simopoulos (2001).

The current problem of Western diets is not the presence of omega 6, but in its imbalance with respect to the content of omega 3 fatty acids. The dietary reference intake (DRIs) for Omega-6 and Omega-3 fatty acids, suggested at the workshop sponsored by the National Institute of American Health (NIH) in Bethesda, MD in 1999 (Simopoulos, 2000) recommended a decrease in dietary intake of linoleic acid, an omega-6 fatty acid (18:2 n6) to 2% of energy and an increase in the amount of alpha-linolenic acid (18:3 n3), an omega-3 fatty acid for 1 % of energy, providing a 2/1 ratio of omega-6: omega-3, whereas the ratio in the United States is now 16,8/1 and Western Europe and North are located in 15/1 (Simopoulos, 2001). However, there are differences on this recommendation, as Sacks and Campos (2006) argue that the omega 6 would have beneficial effects on cardiovascular disease and would help as anti-inflammatory. However, mainstream literature highlight the benefits of omega 3 especially for this purpose as well as the lowest ratio of omega 6/3 (Simopoulos, 2008; Cordain et al, 2005; Simopoulos, 2001, Simopoulos, 2000). Simopoulos (2002, 2001) compares the Greek diet with the Paleolithic period through simulations based on populations that live by hunting and fishing (Figure 7), which suggests an omega 6/3 ratio close to 1, in addition to lower intake total fat, lower intake of saturated fat, almost no trans fats such as eating a more healthy and balanced.

This is evidenced by lowest rates of cardiovascular disease, cancer, infant mortality and highest life expectancy in Greece, especially on the island of Crete (Simopoulos, 2008; Simopoulos and Visioli, 2000; Simopoulos, 2001; Simopoulos and Robinson, 1999; Keys, 1970), where the diet consists largely by Mediterranean plants and moderate portions of yoghurt and cheese from goats and sheep reared on pasture.

Even with 37% of dietary energy from fat, Greek life expectancy exceeds that of Japan, which has low-fat diet, while the milk, cheese and meat in Greece are generally from small ruminants (goats and sheep) fed on temperate grass, which are rich in omega 3 (Simopoulos, 2001) and provide more CLA (Martin and Jenkins, 2002) than animals in confinement/indoor. However, one should bear in mind the association of a healthy diet with a less stressful lifestyle of the islanders, in the standard Western urbanized since the occurrence of carcinogenesis is multifactorial in origin.

Changes in FA profile of sheep and goat's milk fat may not differ substantially from the pattern described for cow milk. If there are differences between ruminants, then goats appear to be the exception rather than ewes or cows (Chilliard and Ferlay, 2004). To increase levels of polyunsaturated fatty acids (PUFA) in milk, dietary PUFA oil intake and factors which decrease their hydrogenation in the rumen have been successful for goat milk, such as trapping of FA in vegetable cells, high forage/concentrate ratios, and PUFA rich protected fat (Chilliard and Ferlay, 2004; Sanz Sampelayo et al., 2004; Park et al., 2007). Generally, vegetable oils are more effective than whole or treated oilseeds (Chilliard and Ferlay, 2004; Haenlein, 2004). Milk yield and fat and protein content responses to lipid supplements are, however different between the bovine, goat and ovine species. Milk fat content increased in general without a change in milk yield in dairy goats, whereas the response in cows was an increase in milk yield and either an increase or a decrease in milk fat content (Chilliard et al., 2004; Ferlay et al., 2006; Park et al., 2007). Comparisons between studies show that milk fat from diets including maize silage is richer in SCFA and linoleic acid, but poorer in alpha-linolenic acid compared with grass silage or pasture diets (Chilliard and Ferlay, 2004). When compared with concentrate diet, pasture, especially at earlier growth, decreased milk FA with a putative negative effect (12:0 to 16:0) and increased those having a potential interest for human health (trans-11,cis-9-18:2 and cis-9,cis-12,cis-15-18:3), respectively. According to Ferlay et al. (2006), compared with fresh grass, grass silages have disadvantages in mono- and polyunsaturated fatty acids levels of milk. To goats feeding with lowconcentrate: forage, linseed oil (flaxseed) have a higher impact about PUFA omega-3 than



the crudelinseed (Chilliard and Ferlay, 20004), in addition decreased milk linoleic acid, MCFA and AI percentages as well as to cows. Decreasing forage:concentrate ratio to 45:55 depressed milk fat percent, increased protein content, milk yield and weight gain, while decreasing eating and rumination time (Haenlein, 2002).

## **b) Short and Medium-Chain Fatty Acids**

Fatty acids (FA) with different chain lengths are submitted to different routes in human digestion and metabolism. When the milk and their fat is ingested, short and medium chains triglycerides until 14 carbons, for the most part are not incorporated into body fat, in contrast to long chain fatty acids. Therefore, mostly short-chain FA do not contribute to obesity as well as some long chain FA, mostly, or to problems related to heart disease (Greenberger and Skillman, 1969), but there are medium-chain fatty acids considered hypercholesterolemic, as the myristic (C14:0) and palmitic (16:0) (Osmari et al, 2011), and other abundant trans fatty acids in margarines.

Milk fatty acids have a dual origin: they are either taken up from plasma lipoproteins (60% of the fatty acids secreted in milk, or they are synthesized *de novo* in the mammary gland from acetate and 3-hydroxybutyrate (Chilliard and Ferlay, 2004).

The main metabolic pathway involves two key enzymes: acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS). The occurrence of interspecific differences between cattle and goats have been established for composition of milk lipids about the way that the acetate supplied by rumen bacteria is used by the mammary gland to synthesize fat from milk, whereas goat's milk has more medium chain fatty acids than cow's milk (Parkash and Jenness, 1968). It is also distinct the way which FA are selected metabolically to access the glycerol molecule in the synthesis of fat, between goats and cows (Jenness, 1980). In addition to interspecific differences, the composition of milk fat is markedly influenced by diet, both for the cow, goat or human, although the composition of milk from ruminants is less affected than in monogastric animals.

Classically, MCFA, SCFA (C4:0–C10:0) remain unchanged or only slightly reduced by increased lipid supplementation in the diet or body lipid mobilization, consistent with data of Chilliard and Ferlay (2004) and Osmari et al (2011). The main fatty acids stored as triglycerides in ruminant adipose tissue are 16:0, 18:0 and 18:1n-9. Thus, lipid mobilization which occurs in

early lactation and when the energy balance is negative induces a sharp increase in stearic, oleic and polyunsaturated acids, inhibiting de novo synthesis of fatty acids with less than 16 carbons in milk (Chilliard and Ferlay, 2004). The total concentration of free fatty acids (FFA), particularly short chain fatty acids (C4:0, C6:0 and C8:0) in milk, has extreme impact about cheese goat flavor and aroma. FFA is a measure of the lipolysis degree in milk, including cheeses, being highly correlated to the frequency of tartaric taste, bitter and sour (Eknaes et al, 2005), reducing the product acceptance by consumers (Soryal et al, 2005).

In the case of goat milk, C6:0-C9:0 FFA, highlighting the branched-chain fatty acids C9:0 and C10:0, provide the typical goat flavor in fresh milk, because they are more abundant in milk from small ruminants than in bovine milk (Delacroix-Buchet and Lamberet, 2000). When the rate of lipolysis in milk is high, the unpleasant taste caused by butyric acid (C4: 0) may appear free (Lamberet et al., 1996), but on the other hand, they claim that it maybe help to prevent tumors inhibiting the urokinase, which is associated with the entry of malignant cells in cell substrate (Youngand Gibson, 1994).

In this sense, to resolve the fact that many consumers do not prefer goat's milk and its products because of its flavor despite its benefits, Abou-Ayana andEl-Deen (2011) successfully improved the sensorial properties and storage of a Labneh yogurt, typical from Middle East through addition of aromatic oils such as chamomile oil to goat's milk or partial replacement of milk fat with sunflower oil or corn oil, which can be effected during the milk processing.

### **c) Conjugated Linoleic Acid (CLA): Origin and Importance**

Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid (CLA, C18: 2, c-9, C-12) with two conjugated unsaturated double bonds with carbon atoms arranged in five possible positions of acid octadecadienoic: cis9, cis11 or linoleic acid (CLA); trans9, trans 11; cis9, trans11 or ruminic acid (RA); trans9 cis11; cis10, cis 12; trans10, trans 12; cis10, trans12; trans10, cis12 (Kelly et al, 1998).

The isomer profile of hydrogenated vegetable fats is very different during hydrogenation of vegetable fats a range of trans-monounsaturated FA (TFA) are principally formed (e.g. trans-9C18:1, elaidic acid), while the main TFA in milk fat is trans-11,C18:1, vaccenic acid (TVA) (Park et al, 2007).

More than 90% of the total CLA-isomers are in the form of ruminic acid, found abundantly in the products from ruminant, it is originating of microbial isomerization (Martin and Jenkins, 2002), the ruminic acid(RA).

CLA is formed as an intermediate during the biohydrogenation of LA to stearic acid by *Butyrivibrio fibrisolvens* and other ruminal bacteria or from the endogenous conversion of transvaccenic acid, the trans-11, C18:1 (TVA) by  $\Delta^9$ -desaturase in the mammary gland (Bauman and Griinari, 2001; Khanal and Olson, 2004). The search of higher concentrations of CLA in milk and other animal products is due to their antimutagenic properties, anticarcinogenic (Martin and Jenkins, 2002; Ohtsu et al, 2005), antiteratogenic, hypocholesterolemic, their role as trigger the immune response to Arteriosclerosis, prevention of obesity and diabetes, as well as acting to inhibit oxidation (Parodi, 1999; Kritchevsky, 2000, Kim and Park, 2003; Khanal and Olson, 2004). Ohtsu et al (2005) suggest that CLA may be useful for chemoprevention of various types of tumors, in contrast to linoleic acid.

In this line, two types of experiments have demonstrated chemopreventive properties in animal models of cancer and the antiproliferative effects in several types of cancer cells:

- In vitro: Inhibition of proliferation of human breast cancer cells, colon, prostate and lung has been attributed to CLA supplementation (Tanmahasamut et al, 2004);
- In vivo: The dietary supplement of CLA has been reported to reduce the incidence of breast and colon tumors in rats (Kim and Park, 2003) and antiproliferative effects in prostate cancer (Ochoa et al, 2004; Ohtsu et al, 2005).

The ability of CLA to attenuate inflammation and cell proliferation by inhibition of these transcription factors could be the key in their ability to limit the development of cancer (Ohtsu et al, 2005).

There is also a large market demand for synthetic CLA, seeking a miraculous weight loss, which is why, according to State Department of Health of the Federal District (SESDF, 2006) of Brazil, has not yet been released to market any supplement conjugated linoleic acid, because the supplement label recommended daily intake of 3-6 g (SESDF, 2006), while the intake of CLA supplementation over 6 g/day can cause brain damage and even signs of insulin resistance, according to the Department. Thus, it is preferred stimulated intake of CLA in foods such as meat and dairy milk to supply in the appropriate and safe amount, strengthening the search for higher

yields of CLA animal by artificial supplementation in monogastric or by inclusion of protected fats and pasture for ruminants, while the latter two are the main source of CLA for humans. Several factors seem to affect the CLA in milk and meat from ruminants and consequently a large variation occurs between the milk and meat samples collected from a group of animals fed the same diet and under similar circumstances.

Factors affecting the production of CLA can be divided into three main categories, according to Khanal and Olson (2004):

- a. Related to diet;
- b. Related to the animal;
- c. Related to post-milking and processing.

Among the factors related to diet, it may be noted the provision of pasture>hays>silages compared to the diet with high concentrate to increase the CLA.

Figure 8 illustrates an example from trial this author, about the lipidic nutritional superiority, whereas milk profile from Boer x Saanen crossbreed goats on pasture supplemented with hay mulberry are better than those supplemented with corn silage.

The findings of Chilliard and Ferlay (2004), Ferlay et al. (2006) and Osmari et al (2011) are consistent about the disadvantages of maize silage against fresh temperate grass or hay based-diets with regard to CLA, UFA, desirable fatty acids (DFA) and atherogenic index (AI). Khanal and Olson (2004), in their literature review, point out that various researchers have shown an increased CLA content in milk and meat from ruminants by grazing on pasture (Loor et al., 2002), supplementing total mixed rations (TMR) containing 50% forage and 50% concentrate with plant oils or oil seeds, and supplementing TMR with fish oil.

Especially the roughage, with added oil, resulted in higher values of CLA compared to animals fed with diet added oilseeds, and both an increase of CLA in relation to the group only with TMR in both goats and cattle.

In order to 70:30 concentrate: forage ratio feeding based on soybean grain or flaxseed did not increased levels of CLA in goat's milk (Chilliard, 2003), results in part explained by the importance of diet and its effect on pH, to be above 6.0, because usually, low proportions of forage: concentrate diet caused a reduction in rumen pH, and the complete biohydrogenation of linoleic acid to stearic occurs below 6.0, with a negative effect on the concentrations of both ruminal CLA and TVA, its precursor (Martin and Jenkins, 2002).

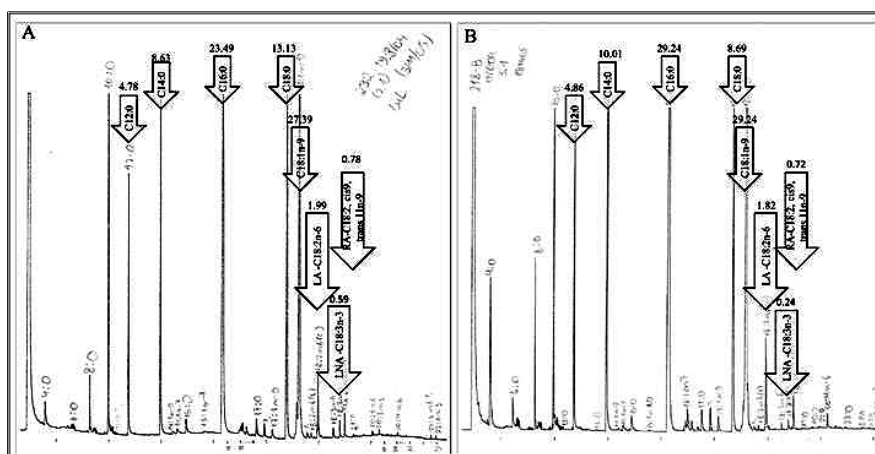


Figure 8. Example of two chromatographic profiles (gas chromatograph with FID), as percentage of total milk fatty acids, of goats receiving low-concentrate (0.9% of initial BW) and supplemented at libitum with mulberry hay (A) or corn silage (B), with 5 weeks lactation average on pasture-based diets (*Cynodon spp.* and *Avena strigosa*), in trial conducted by Osmari during winter of 2004 year.

In practice, the CLA production may be positively associated with the grazing animal, because of their greater expenditure of time to select the food, the lower pass rate as a function of effective fiber of pasture, which stimulates production of saliva buffering, resulting in ruminal pH higher than that of confined animals with low levels of forage in the diet. The diversity of forage species, valued at grazing can also increase the content of CLA in milk fat, which can be positively influenced by lower maturity and higher altitudes of the region.

According to the work of Collomb et al (2002), held in the temperate region, which indicates that highland pastures provide more CLA, but the effects of several varieties of grasses and legumes in the content of CLA in milk remain unclear and may be more related to the composition of individual fatty acids of forage than with anything else (Khanal and Olson, 2004).

Indeed, one problem encountered with fat supplementation in related to specie, in dairy cows and ewes is that the milk protein content is most of times reduced, thus altering coagulation properties. However, this negative effect does not exist in goats (Chilliard and Ferlay, 2004; Park et al, 2007). Furthermore, the clear positive effects of almost all types of fat supplementation on milk fat content could be useful to solve the technological problems of the goat cheese industry which are linked to the so-called “inversion of percent-

ages syndrome” during the spring and summer period (Chilliard and Ferlay, 2004). This effect could be related to the combined effects of lactation stage, day length (dilution effect, due to stimulation of milk yield; Chilliard et al, 2003; Ekanes et al. 2005), and/or nutritional factors.

Summarizing the differ responses to fat supplementation according to the species (Chilliard et al, 2003):

- Milk yield increases in midlactation dairy cows, but not in goats and ewes;
- Milk fat content (and fat secretion) sharply increases in dairy ewes and goats, but not always in dairy cows in which it could often either decrease or not change;
- Milk protein content decreases in dairy cows and ewes, but not in goats. Milk protein secretion decreases in milking ewes, but does not change in dairy cows and goats.

In relation to goat breeds, the milk from Nubian goats resulted in a much higher cheese yield, a lower oleic acid concentration and a lower unsaturated fatty acid concentration than that from Alpine goats (Soryal et al, 2005). The researchers relate also more abundant unsaturated fatty acids (particularly C18:1, and C18:2) in cheese of Alpine milk than Nubian milk in this study would indicate significant quantities of *cis*-9, *trans*-11, and *trans*-9, *trans*-11 C18:2 (conjugated linoleic acids, CLA). Related to post-milking and processing, fresh goat's cheese from grazing system represents a higher source of CLA for human consumers than fresh cow's cheese those same system, offering from 156.6 to 222.6 mg/ 100 g of sample, in agreement to Nieuwenhove et al (2009). The researchers found CLA level averaged 0.85 and 0.96 in milk and 0.76 and 1.04 g/100 g of fatty acids in cheese of cow and goat, respectively, both fed on natural grassland from northwest Argentina.

Lastly, care should be taken in causal studies, since the origin of carcinogenesis and cardiovascular diseases is multifactorial, and is need update for inclusion of CLA in equations of atherogenic (AI) and thrombogenic indices (TI) suggested by Ulbright and Southgate (1991), but in according to research in health human. The bioactive peptides also should be better studied to establish relationships with lipids of interest.



Figure 9. Example of crossbreed Boer x Saanen crossbreed goats on pasture, South Brazil.

Thus, there is a challenge to improve the ratio of hypocholesterolemic and hypercholesterolemic fatty acids and decrease the atherogenic index to human health, which in the next few years can become a tool in the promotion of production systems of dairy goats based on pasture and supplementation.

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*Chapter 3*

## **INFECTIOUS CAUSES OF ABORTION IN GOATS**

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### **ABSTRACT**

The occurrence of abortions in goat herds at a level that significantly affects herd performance is a common clinical problem. The microorganisms that cause abortions in goats are viruses, bacteria, and protozoa. The viruses include caprine herpesvirus 1, border disease, and Akabane virus. The bacteria species include *Brucella melitensis*, *Campylobacter* spp, *Chlamydophila abortus*, *Coxiella burnetti*, *Leptospira* spp, *Listeria monocytogenes*, *Mycoplasma* spp, *Salmonella* spp, and *Yersinia pseudotuberculosis*. The protozoa species include *Neospora caninum*, *Sarcocystis* spp, and *Toxoplasma gondii*. Each cause of abortion is discussed in this chapter, including the etiology, the clinical signs, the diagnosis methods, and the management strategies for the disease.

## INTRODUCTION

The reproductive efficiency success may be affected by infertility and abortions. Abortion in goat herds at a level that significantly affects production is a common clinical problem. Each country or region may have a different prevalence of specific causes of abortion, but usually the most commonly diagnosed causal agents of abortion in goats are from infectious origin. In this complex syndrome, the most prevalent microorganisms are viruses (caprine herpesvirus 1, border disease, and Akabane virus), bacteria (*Brucella melitensis*, *Campylobacter* spp, *Chlamydophila abortus*, *Coxiella burnetti*, *Leptospira* spp, *Listeria monocytogenes*, *Mycoplasma* spp, *Salmonella* spp, *Yersinia pseudotuberculosis*), and protozoa (*Neospora caninum*, *Sarcocystis* spp, *Toxoplasma gondii*).

## VIRUSES

### Akabane Virus

The host range of Akabane virus includes sheep, cattle, and goats. Isolates of the virus have been reported in Africa, Japan, Israel, South Africa, Argentina, Korea, Australia, and the Middle East. The virus is thought to be transmitted to the livestock through different *Culicoides* species (mainly *Culicoides brevitarsis*) (Washburn and Streeter, 2004; Givens and Marley, 2008). Experimentally infected lambs developed hydrocephaly when exposed to the Akabane virus on days 30 to 36 of gestation. Non-pregnant goats do not show clinical signs, whereas pregnant does can appear healthy but abort, have stillbirths or mummified fetuses. Kids that do not die from *in utero* infection are often born with arthrogryposis and/or hydrancephaly that can result in dystocia. Akabane virus has reportedly caused hydranencephaly in addition to other defects, such as arthrogryposis, in the developing fetus of goats after infection of the doe (Inaba et al., 1975; Washburn and Streeter, 2004).

### Border disease virus (BDV)

Border disease is an endemic infection of small ruminants that was first reported in 1959 from the border region of England and Wales (Hughes et al.,

1959). BDV is a togavirus closely related antigenically to Bovine Viral Diarrhea Virus (Washburn and Streeter, 2004). Clinical signs in sheep include barren ewes, abortions, malformations, stillbirths, birth of small weak lambs, and persistent infections of the offspring. Affected lambs can show tremor, abnormal body conformation and hairy fleeces (also known as hairy-shaker-syndrome and fuzzy lambs syndrome) (Smith and Sherman, 1994). BDV has also caused mucosal disease-like lesions in sheep (Monies et al., 2004). BDV infections in pregnant goats cause almost exclusively in abortions and malformations in fetuses and neonates (Nettleton et al., 1998).

Transplacental infection with BDV leads to hydrocephaly, in addition to various other defects of the central nervous system, skeleton, and dermis. Interspecies transmission between ruminants occurs naturally; however, strain variations, differing host responses, and gestational age at which infection occurs all contribute to the diverse clinical manifestation of disease (Washburn and Streeter, 2004). In sheep, developmental disturbances of the cerebellum and dermis accompany commonly hydranencephaly. These accompanying defects manifest clinically in the form of whole-body tremors and an abnormal birth coat with long hairs that stand on end. In kids, rhythmic muscle tremors, predominantly in the hind limbs, have been reported after *in utero* infection (Smith and Sherman, 1994).

## Caprine Herpesvirus 1 (CpHV-1)

CpHV-1 which belongs to the Family Herpesviridae, sub-family Alpha-herpesvirinae, has a characteristic tropism for the genital tract of the goat. In adult goats, CpHV-1 causes late-term abortion without any prior clinical signs (Tempesta et al., 2004; Givens and Marley, 2008). The virus can also cause vulvovaginitis or balanoposthitis and, less frequently, respiratory disease (Tempesta et al., 2004). In goat kids aged 1-2 weeks, CpHV-1 is responsible for a generalized disease characterized by high morbidity and mortality, which shows clinical signs of pyrexia, conjunctivitis, oculonasal discharge, dyspnoea, abdominal pain and weakness leading to death within 1-4 days (Smith, 1997). A significant post-mortem finding in kids consists of erosive, ulcerative and necrotic lesions of the mucosa throughout the gastrointestinal tract (Roperto et al., 2000). A survey showed that CpHV-1 was the only viral agent identified as a cause of abortion in goats from California-USA accounting for 4% of the total diagnostics (Moeller Jr, 2001). After primary infection, CpHV-1 induces a latent infection that is very difficult to reactivate. Under natural conditions,

reactivation has been observed only in coincidence with oestrus in does with low neutralizing antibody titers, stress or immunosuppression (Tempesta et al., 2004). Following reactivation, the virus can be shed via the respiratory or genital route. Subsequent pregnancies are not affected (Givens and Marley, 2008).

Caprine herpesvirus has a negative effect on the reproductive system and especially on pregnancy. The economic impact of the infection which it causes, returns to services and abortions, justifies the appropriate prophylaxis including the immunization of goats belonging to a CpHV-1-infected flock. Vaccination of seronegative animals protects them from clinical signs of infection, reduces viral titres and shortens the duration of viral shedding, and thus appears to be a promising tool for the control and, subsequently, eradication of CpHV-1 infection (Tempesta et al., 2001).

## BACTERIA

### *Brucella melitensis*

Brucellosis is a complex disease, due to the variety of *Brucella* species involved that, although having a species-specific disease, can sometimes cross-infect. *B. melitensis* is the main etiological agent of brucellosis in sheep and goats. It is also the primary agent responsible for human brucellosis, known as Malta fever (synonymous with undulant fever, Gibraltar fever, and Mediterranean fever). Abortion and infertility are the predominant clinical signs in small ruminants. Does might show fever, depression, weight loss, diarrhea, mastitis, lameness, and birth of weak kids. Bacterium is shed in milk, urine, feces, and for 2–3 months in vaginal discharge. Its incidence is common in countries at the south and east of the European Union Africa, Mexico, the Middle East, India, Pakistan and parts of South America (Givens and Marley, 2008; Blasco and Molina-Flores, 2011).

The transmission to humans of *B. melitensis* from infected animals may be either direct or indirect. The mechanism of direct transmission involves the respiratory, conjunctival, and cutaneous routes, which is particularly prominent in people that work with infected goats or sheep (Wallach et al., 1998). Human brucellosis is primarily an occupational disease; professions with direct exposure to livestock (farmers, butchers, veterinarians, laboratory personnel, and so forth) are those at higher risk (Blasco and Molina-Flores, 2011). The consumption of unpasteurized goat milk or cheese may transmit indirectly



to humans. The disease in humans is characterized by chills, fever, headache, sweats, and rachialgia (Wallach et al., 1998); demonstrating the difficulties in recognizing a disease that, although grave, lacks pathognomonic symptoms and is thus underreported (WHO, 2005).

The diagnosis of brucellosis includes direct (bacteriological and molecular) and indirect (serological and allergenic), and cell-mediated immunity based diagnosis. The bacteriological diagnosis of *B. melitensis* can be made by means of the microscopic examination of stained smears from vaginal swabs, placentas or aborted foetuses (Stamp's method). Thus, taking vaginal swabs and milk samples is the best way to isolate *B. melitensis* from sheep and goats (Garin-Bastuji et al., 2006). The spleen and lymph nodes (iliac, supramammary and prefemoral) are the best sites for obtaining samples for isolation during the post-mortem examination (Marín et al., 1996). PCR assay has been shown to be an effective method for detecting DNA from different microorganisms and provides a promising option for diagnosis of brucellosis (Garin-Bastuji et al., 2006). Recently, a new method has been described for fingerprinting *Brucella* isolates based on multi-locus characterization of a variable number, 8-base pair, tandem repeat. The technique is highly discriminatory among *Brucella* species or strains (Bricker et al., 2003). Indirect detection methods consist of the Rose Bengal test, complement fixation test, enzyme linked immunosorbent assays (ELISA), native hapten gel precipitation tests, and fluorescence polarization assay. However the isolation of *Brucella* bacteria is the most reliable and the only unambiguous method for diagnosing animal brucellosis (Alton et al., 1988).

It seems difficult to rely solely on hygiene measures to control brucellosis, due to the fact that extensive farming, transhumance and nomadism are widespread in various regions worldwide. However, whenever possible, disinfection of infected premises and destruction of abortion products, as well as removal of aborted females, contribute to reduce the contamination level, thus allowing vaccination to be more effective (Benkirane, 2006). The live-attenuated *B. melitensis* Rev-1 vaccine is the only vaccine available, and it has been proved to be useful for prevention of infection in sheep and goats. However, when administered by the classic subcutaneous method (individual doses of  $1 \times 10^9 - 2 \times 10^9$  CFU), a long-lasting serologic response is induced, which makes an eradication program based on combined test and slaughter impractical. When the same vaccine is administered by the conjunctival method (at the same dose, but applied by conjunctival instillation in a smaller volume), the immunity conferred is similar to that induced by the classic subcutaneous method, but the serologic responses evoked are significantly

reduced, making this program fully compatible with the application of an eradication program based on vaccination combined with trial and slaughter (Benkirane, 2006; Blasco and Molina-Flores, 2011). Occasionally goats are infected by *B. abortus* due to direct contact to infected cattle or as the result of unaware vaccination with live strains of the microorganism (Smith and Sherman, 1994).

### ***Campylobacter* spp**

*Campylobacter jejuni* and *C. fetus subsp fetus* are becoming the predominant species associated with ovine abortion in the United States (Menzies, 2011). Campylobacteriosis is rarely a reported cause of abortion in goats (Smith and Sherman, 1994). The bacterium can reside in the intestinal tract and gall bladder of sheep and goats, as well as of many other animal species including dogs, poultry, and wild birds. Carrion-eating birds, such as crows and seagulls, may serve as a mode of transmission between flocks (Ogden et al., 2009). *C. jejuni* is well known as a human pathogen, and it is responsible for mild diarrhea and occasionally septicemia, abortion, arthritis, and Guillain-Barré syndrome in humans (Smith, 2002). Does may exhibit late abortions and a mucopurulent vaginal discharge. Kids may also be stillborn, or born weak and undersized if infected closer to term. Fetuses are aborted, usually about 3 days after death, and fetal livers may appear swollen with areas of target-shaped necrosis. The placenta is edematous, with necrosis of the cotyledons. The does do not show signs of systemic illness, but may have transient diarrhea (Givens and Marley, 2008; Menzies, 2011).

Use of antimicrobials in the pregnant flock is usually recommended in an outbreak of campylobacteriosis abortion, and, depending on how spread out parturition is and at what stage of pregnancies the disease is diagnosed, it may be effective in reducing abortions during an outbreak (Menzies, 2011). In a recent study, most of the strains of *C. jejuni* isolated were found to be of a single genetic clone. This clone is highly resistant to tetracycline drugs, indicating that the use of this antimicrobial in its control is contraindicated. The same workers found that all 74 *C. jejuni* isolates studied were susceptible to tilmicosin, florfenicol, tulathromycin, and enrofloxacin, whereas 97% were sensitive to tylosin, suggesting that those antimicrobials may be effective in managing a flock outbreak (Sahin et al., 2008).

In the United States and Canada, a commercial vaccine is available. The recommendation is to vaccinate the flock initially, using a primary series 60 to

90 days apart, starting before breeding, then to revaccinate annually, again before breeding. In the United States only, another vaccine, which recommends specifically that the booster be given in mid-to late gestation, is available. Based on evidence listed in the product insert, it seems useful in preventing campylobacter abortions. In New Zealand, a similar attenuated combination product is available, with the primary series and booster vaccination being administered before breeding (Menzies, 2011).

### ***Chlamydophila abortus***

This organism is one of the most important causes of abortion in sheep and goats worldwide, except for Australia and New Zealand. *C. abortus* is a gram-negative intracellular organism that has 2 states: elementary bodies inside the cell and reticular bodies as the environmental form (Smith and Sherman, 1994; Menzies, 2011). In females, this bacterium has tropism for the placental tissue, whilst in male animals the infection can cause orchitis and seminal vesiculitis, resulting in shedding of the organism in semen (Nietfeld, 2001). Most animals become infected by ingestion of organisms in contaminated food or water, licking does contaminated with placental fluids or tissues, or inhalation of aerosols created in the infected environment (Nietfeld, 2001). The organism can also reside in pigeons or sparrows and be transmitted via ticks or insects (Givens and Marley, 2008).

In the initial outbreak, up to 30% of sheep and 60% of goats can abort or give birth to stillborn and weak offspring. Thereafter, yearly abortion rates of 5% to 10% with occasional flare-ups affection recently added animals are typical. In a survey of 211 cases of caprine abortion, *C. abortus* was responsible for 46.15% (n=30) of the bacterial abortion causes in the state of California (Moeller Jr 2001). Goats can abort at any stage of pregnancy, but most abortions are during the last 2 to 3 weeks of gestation (Nietfeld, 2001). In most cases, abortion is the only clinical manifestation of *C. abortus* infection in goats, but concurrent respiratory tract disease, polyarthritits, conjunctivitis, and retained placentas have been reported (Givens and Marley, 2008). The placental membranes may present variable degree of necrotic damage, although often the majority of the placenta have thickened red inter-cotyledonary membranes and dark red cotyledons with a creamy-yellow colored exudate on the surface (Longbottom and Coulter, 2003). Fetuses may be aborted necrotic, well preserved, or, rarely, mummified. Weak and stillborn kids are often seen. The cause of fetal death is believed to be the severe

placentitis, which causes hypoxia and retardation of fetal growth. Females infected as lambs/kids, before mating or at the final stage of gestation, do not usually abort until the following pregnancy. After abortion, infected ewes/does are immune and unaffected in subsequent pregnancies, but they can shed the organism in vaginal secretions during estrus, thus infecting other animals that may be pregnant at the time (Stuen and Longbottom, 2011).

If *Chlamydophila* abortion is suspected to be present in a flock/herd, the administration of a long-acting oxytetracycline preparation (20 mg/kg body weight intramuscularly) will reduce the severity of infection and losses resulting from abortion. It is essential that treatment is given soon after the 95<sup>th</sup> to 100<sup>th</sup> day of gestation, the point at which pathologic changes start to happen. Further doses can be subsequently given at 2-week intervals until the time of lambing. Although such treatment reduces losses and limits the shedding of infectious organisms, it does not eliminate the infection nor reverse any pathologic damage already done to the placenta. Thus, abortions or delivery of stillborn or weakly lambs can still occur, and the shed organisms are a source of infection for other animals (Longbottom and Coulter, 2003; Stuen and Logbottom, 2011). In chronically infected flocks, feeding levels of 250 to 500 mg of the oxytetracycline per animal daily, starting 60 days before the first expected lambing date has often been recommended. Higher levels are usually fed during an outbreak of abortion, and lower levels are fed prophylactically. However, recommendations on dosage and length of time can vary and there is no published information on true efficacy (Menziez, 2011).

In an outbreak, the first goal is to limit the spread of infection by removing affected animals quickly and isolate from the main flock for about 3 weeks, by which time they should stop shedding chlamydiae (Nietfeld, 2001). All dead fetuses, placental membranes, and bedding should be carefully disposed of; parturition pens must be cleaned and disinfected (Longbottom and Coulter, 2003). In most of Europe, there is currently available an attenuated vaccine based on a temperature-sensitive mutant strain (*C. abortus* strain 1B) that is available from 2 commercial companies. The vaccines must be administered at least 4 weeks before mating and cannot be used in combination with antibiotic treatment. Inactivated vaccines can also be prepared from organisms grown in hens' eggs or cell culture. These vaccines are safe for administration during pregnancy (Kadra and Balla, 2006; Stuen and Longbottom, 2011).

Although rare, the greatest threat of human infection is to pregnant women, for whom the outcome of infection in the first trimester of pregnancy is likely spontaneous abortion; later, infection causes stillbirths or preterm

labor. Several cases of abortion, puerperal sepsis and shock, including renal failure, hepatic dysfunction, and disseminated intravascular coagulation, as well as death have been reported. Most cases are associated with direct exposure to infected sheep or goats (Longbottom and Coulter, 2003; Stuen and Longbottom, 2011).

### ***Coxiella burnetii***

*Coxiella burnetii*, aerobic intracellular organism, is the etiological agent of Q fever. Although *Coxiella* was historically considered being a Rickettsia, gene-sequence survey now classifies the genus *Coxiella* in the order Legionellales, family Coxiellaceae, with *Rickettsiella* and *Aquicella* (Stuen and Longbottom, 2011). The bacterium can infect a wide range of hosts, including ruminants (cattle, sheep, and goats), swine, cats, dogs, wildlife, rodents, birds, ticks, and humans. *C. burnetii* is shed in birth fluids, vaginal secretions, feces, and milk. This shedding can persist for weeks to months and the organism can survive for months to years in the environment, making infected animals a significant risk for human infection. Sheep and goats become infected most commonly from mucous membrane contact with aborted materials but also from vaginal secretions or fluids and membranes from a normal parturition of flock mates, birth products and excretions of other species of animals, contaminated air, dust, manure, bedding, and semen from infected males. Ticks may also shed the organism and contaminate the wool (Menzies, 2011).

In animals, *C. burnetii* infections are usually asymptomatic; except for abortion, stillbirth, and the delivery of weak offspring, clinical signs in ruminants are limited. However, *C. burnetii* may induce pneumonia, conjunctivitis, and hepatitis (Arricau-Bouvery and Rodolakis, 2005). The abortion rate can range from 3% to 80% of pregnant females. High abortion rates are rarely observed, although abortion storms in some herds have been described (Sanford et al., 1994). Stress, resulting from overcrowding or poor nutrition, may play a decisive role in an infected goat aborting. In the majority of cases, abortion or stillbirth occurs at the end of the gestation period, without specific clinical signs, only when placental damage has been severe. Aborted fetuses appear normal, but infected placentas exhibit intercotyledonary fibrous thickening and discolored exudates that may be mineralized (Stuen and Longbottom, 2011).

Current alternatives to diagnose *C. burnetii* infection in ruminants include serologic analysis, organism isolation by cell culture or live animal inoculation, and immunohistochemical and PCR-based detection (Stuen and Longbottom, 2011). Ancillary diagnostic methods include placental smear or impression of placentas stained by a modified Ziehl-Nielsen procedure; complement fixation test, enzymelinked immunosorbent assay (ELISA), and a fluorescent antibody test (Kovacova et al., 1998).

If Q fever is suspected, aborting animals and other animals in late pregnancy should be treated with tetracycline. The regime consists in 2 injections of oxytetracycline (20 mg/kg BW) during the last month of gestation, although this treatment does not entirely suppress abortions and shedding of *C. burnetii* at parturition (Stuen and Longbottom, 2011). Others authors stated that shedding is not significantly affected by the prolonged use of antimicrobials in an infected flock/herd (Menzies, 2011). Application of biosecurity precautions is recommended to reduce the spread of the organism, specifically to reduce risk to humans. These include burying or incinerating placentas and aborted fetuses, composting manure for at least 5 months before spreading, spreading manure only on still days, disinfection of lambing/kidding areas after careful removal of manure, wearing N95 masks when working with animals or moving manure, wearing gloves or disposable plastic sleeves when assisting with lambings/kiddings, and maintaining biosecurity regarding protective clothing (Berri et al., 2007; Menzies, 2011).

In humans, Q fever can lead to an acute disease (self-limited febrile illness, pneumonia, or hepatitis) or to a chronic disease, mainly endocarditis in patients immunocompromised or suffering from valvulopathy, or abortions and stillbirth in pregnant women. Humans become infected mainly by inhalation of contaminated aerosols or dusts containing *C. burnetii* shed by infected animals. Oral transmission of the bacteria (via contaminated dairy products), although considered as negligible is also reported in the literature, as well as the risk of vertical and sexual transmission (Guatteo et al., 2011). In the Netherlands, since 2007, Q fever has become a public health problem with 2357 human cases notified in the year 2009, human cases still being notified in 2010. Epidemiological investigation conducted reports that abortion waves on dairy goat flocks were likely to be the source of human infection, affecting mostly people living near to these dairy goat flocks (Van der Hoek et al., 2010).

### ***Leptospira* spp**

Goats and sheep are considered less susceptible to leptospirosis than other domestic farm animal species (e.g. cattle). Leptospirosis in small ruminants may appear in an acute form, with pyrexia, anorexia, depression, jaundice, and anemic or hemorrhagic syndromes (Smith and Sherman, 1994). Nevertheless, they may develop the chronic form of the disease; which is characterized by impaired fertility, neonatal deaths, abortions mainly in the last trimester, and decreased milk production, causing significant financial losses (Lilenbaum et al., 2008). Besides, small ruminants are able to develop chronic renal infection and maintain persistent leptospiuria, disseminating bacteria to other animal species as well as to humans (Lilenbaum et al., 2009). Humans should avoid contact with aborted tissues and infected urine, due to the zoonotic potential. In several states of Brazil, the disease has been reported for approximately 40 years. In Rio de Janeiro state, leptospirosis is a major infectious disease affecting the reproductive performance of dairy goats. In a recent study, 11.1% of goats tested were seroreactive, predominantly due to serovar Hardjo (Lilenbaum et al., 2007).

The standard serological diagnosis of leptospirosis is the microscopic agglutination test, which is recommended for the diagnosis on a herd-screening basis. It relies on the seroreaction with live bacteria representative of local serovars. Nevertheless, the correlation of serology with the presence of bacteria in the kidneys or in extra-renal locations is not evident, and the direct detection of the organism is necessary to determine reliably carriers, as part of an effective control program. Efforts to identify carriers are directed towards the detection of the agent or its DNA in urine and other tissues. Available techniques include dark field direct microscopic examination, bacterial isolation, and the detection of leptospiral DNA by polymerase chain reaction (Lilenbaum et al., 2008; 2009). Since leptospiral cultivation is laborious, time-consuming, and contamination of cultures may occur, other approaches are welcome to identify carriers of leptospires. PCR has been used to detect *Leptospira* spp. in clinical specimens as bovine urine and vaginal fluids/semen of goats and sheep (Lilenbaum et al., 2008), with encouraging results.

In endemic regions, vaccination two to four times a year with polyvalent bovine vaccine is indicated. Others control measurements include separation of livestock species, reduction of the rodent population, clean water availability, and administration of tetracycline (300-500mg/animal/day) from the mid gestation to parturition (Smith and Sherman, 1994).

### ***Listeria monocytogenes***

Listeriosis caused by *Listeria monocytogenes* (a nonsporulating, facultative, anaerobic gram-positive rod) is an infectious disease affecting a wide range of mammals, including ruminants, monogastric animals and humans. In ruminants, the main clinical features are encephalitis, keratoconjunctivitis/uveitis, septicaemia, abortion, mastitis, myelitis and gastroenteritis (Brugère-Picoux, 2008).

Animals may be exposed to the organism by direct contact with infective material from same or other animal species. Feces, urine, aborted fetuses, uterine discharge and milk should be considered as infectious, but most cases of listeriosis arise from the ingestion of contaminated food, notably silage. Lambs and kids may acquire septicaemic listeriosis from congenital infection or from contamination on the mother's muddy teat or contaminated milk. In pregnant ewes/does, bacteraemia leads to invasion of the placenta and fetus within 24 hours. Subsequently, abortion occurs 5–10 days later, and finally metritis can be established. Infection at late pregnancy can lead to stillbirths or birth of offspring that rapidly develop a fatal septicaemia. Incubation periods vary with the pathogenesis of the disease. Thus, 1–2 days are typical for septicaemic disease or gastrointestinal form, 2 weeks for abortion and longer for the encephalitic form (4–6 weeks). Abortion usually occurs during the last month of gestation accompanied by fever, depression, endometritis and/or blood-stained vaginal discharge may be present for several days (Low and Donachie, 1997; Brugère-Picoux, 2008).

Antibiotics must be administered for a prolonged period, because recovery can use as long as one month. *Listeria* is susceptible to the majority of antibacterial agents currently available, with the exception of cephalosporins. Ampicillin and gentamicin have been reported as the treatment of choice for listeriosis, but other antibiotics can also be used, like procaine penicillin or tetracycline. Vaccines of attenuated or killed bacteria have been used to protect livestock and can reduce annual incidence of the disease, but the effectiveness of these vaccines still requires further studies. After abortion, animals may become chronic carriers and should be culled from the flock. Cases of listeriosis are invariably associated with high rates of environmental contamination (hay, silage, rotten vegetation), but complete elimination of *Listeria* is unrealistic. Finally, care should be taken in the preparation of silage (Smith and Sherman, 1994; Low and Donachie, 1997; Brugère-Picoux, 2008).



### ***Mycoplasma spp***

*Mycoplasma* abortion is often seen as part of a larger clinical syndrome of agalactia, mastitis, arthritis, conjunctivitis, and septicemia in goats and, occasionally, sheep flocks. Members of the mycoides cluster have been implicated in cases of caprine abortion. *Mycoplasma mycoides* subsp. *mycoides* large-colony type has been isolated from the placental cotyledons and joints, lung, brain, and heart blood of aborted caprine fetuses (Rodriguez et al., 1995). Although less frequently encountered *Mycoplasma capricolum* subsp. *capricolum* has also been associated with abortion in goats (Rodriguez et al., 1996). Following abortion does can shed the organism in milk, amniotic fluids, and the placenta (Smith and Sherman, 1994). This organism has been isolated from the vaginal fluid, uterus, uterine fluid, pharyngeal lymph node, axillary lymph node, carpal joint, trachea, tonsils, and external ear canals of that aborted. The organism was also isolated from the placental cotyledons and fetal liver, carpal joint, mouth and skin surface (Ruffin, 2001). Others reproductive problems associated to this microorganism infection include granular vulvovaginitis and balanoposthitis mainly in sheep and rams, respectively (Ruffin, 2001). Diagnostic of *Mycoplasma* abortion is achieved by culture and serum-typing the isolate. Treatment with tetracyclines and tylosin can be useful; however, identification and culling of the infected goats are the best preventive procedures (Smith and Sherman, 1994).

### ***Yersinia pseudotuberculosis***

The bacteria occur in water and environment, being found in various wild and domesticated animals. However, the epidemiology of *Y. pseudotuberculosis* infections is unclear, and the contamination by fecal-oral route is the most common. In multiple outbreaks of yersiniosis by *Y. pseudotuberculosis* in humans, the most common clinical symptoms were abdominal cramps (92%), fever (83%), and articular or back pain (54%). Only about half (52%) of the patients had diarrhea. Symptoms duration was 14 days for 61% of the case patients (Jalava et al., 2004). Abortion and early neonatal death have been reported in goats; when the organism was isolated from placenta and abomasal contents of triplet goat kids, two of which were aborted and one of which died shortly after birth. Necropsy findings in the kids were suppurative placentitis and suppurative pneumonia (Witte et al., 1985).

## PROTOZOA

### *Neospora caninum*

Neosporosis is a leading cause of reproductive failure in dairy and beef cattle in many countries, causing substantial economic losses (Dubey, 2003). *N. caninum* has been sporadically associated with abortion in goats (Eleni *et al.*, 2004) and sheep (Hässig *et al.*, 2003). The role of these protozoa as a cause of natural abortion in small ruminants needs to be further investigated, since their experimental inoculation with *N. caninum* during pregnancy causes a condition remarkably similar to that observed in cattle (Buxton *et al.*, 2002). The infection may cause fetal resorption, mummification, abortion, premature birth and stillbirth. However, most fetuses infected during pregnancy survive and are born congenitally infected but clinically healthy. Rarely, congenitally infected offspring may manifest neurological signs varying from mild ataxia to tetraparalysis (Dubey, 2003). Occasionally adult animals can show clinical signs mainly of encephalitis (Dubey and Schares, 2011).

Histopathological examination of aborted fetuses may show a non-suppurative meningoencephalitis, characterized by perivascular cuffs of mononuclear infiltrates (lymphocytes, monocytes and rare neutrophils) and multifocal necrotic areas associated to gliosis. Tissue cysts, round or oval in shape and 10-20 mm in length, might be found in the brain tissue, occasionally associated to the inflammatory lesions (Eleni *et al.*, 2004). Serologic surveys in different countries show 2-23% prevalence in goats (Dubey and Schares, 2011).

The vertical transmission of *N. caninum* in cattle is efficient, perhaps for several generations; and this behavior is also observed in small ruminants (Buxton *et al.*, 2002). Therefore, one method for preventing the transmission from mother to offspring is culling, but it effective if the prevalence of *N. caninum* is not very high. No drug is known to kill encysted *N. caninum* in bovine tissues (Dubey and Lindsay, 2006). The prevention of horizontal transmission includes the avoiding the females ingesting oocysts-contaminated food and water. The access of dogs and other canids to cattle barns or pasture should not be allowed. The exact form that dogs become infected with *N. caninum* is not known, but the consumption of aborted bovine fetuses probably is not a valuable source of infection. The consumption of placental membranes may be a source of infection in dogs because they feed *N. caninum* oocysts found in naturally infected placentas. Little is known at present regarding the amount of shedding of oocysts by canids in nature, the resistance of the

oocysts, and whether dogs shed oocysts more than once. Until more definitive hosts of *N. caninum* are found, dogs and coyotes should not be allowed to eat aborted fetuses, fetal membranes, or dead calves/lambs/kids. Drugs that prevent transmission of the parasite from the dam to the fetus are unknown, but research is continuing in this area. There is evidence that cattle can develop protective immunity to subsequent neosporosis abortion. This protective immunity seems to be more effective in cows that are subsequently infected with an exogenous source (oocysts) than in cows in which there is a recrudescence of persistent infection. Therefore, to induce protective immunity against abortion in cows with latent infection is a problem (Dubey, 2003; Dubey and Lindsay, 2006; Dubey and Schares, 2011).

Currently, there is a trade *N. caninum* vaccine (Neo Guard) that killed parasites, but there is limited information about its efficiency for preventing abortions (Barkling et al., 2003).

### ***Salmonella* spp**

*Salmonella* spp is an important food-borne pathogen. The consumption of goat's meat or unpasteurised goat's milk and cheese was associated with some outbreaks of infection in humans (Cortés et al., 2006). *Salmonella* infections are zoonotic and can cause enteritis, abdominal pain and abortion in humans. In small ruminants, *S. abortus-ovis* is the most important species responsible for reproductive disturbances. *S. typhimurium*, *S. dublin*, *S. montevideo* and *S. arizona* are also associated to infection and abortion in chronically infected females. Infection in sheep and does can cause abortion, metritis, retained placenta, diarrhea, and systemic illness. Although abortion can occur throughout pregnancy, it is more common at the end of pregnancy. Outbreaks can occur after stressful events such as transportation, overcrowded stables, food privation, and improper use of antibiotics. Prevention includes vaccination and hygienic-sanitary measurements since the bacteria are transmitted by the oral route; whilst treatment consists of antibiotic treatment according to the antibiogram result (Smith and Sherman, 1994; Givens and Marley, 2008).

### ***Sarcocystis* spp**

The genus *Sarcocystis* is composed by more than 100 species that infect a wide variety of animal species. *Sarcocystis* is a cyst-forming protozoan which has an obligatory prey-predator (two-host) life cycle. Asexual stages develop

only in the intermediate host, which is often a prey animal, and reproductive stages develop only in carnivorous, which are the definitive host (Dubey and Lindsay, 2006).

*Sarcocystis* is usually nonpathogenic for the definitive host, and some species of *Sarcocystis* are also nonpathogenic for intermediate hosts. Species transmitted by canids are often pathogenic, but the species transmitted by felids are typically nonpathogenic. *S. cruzi*, *S. capracanis*, and *S. tenella* are the most pathogenic species for cattle, goats, and sheep, respectively; *S. hircicanis* and *S. moule* were also isolated from goats (Dubey and Lindsay, 2006). Clinical signs are commonly present at the acute phase, with the second schizogonic cycle in blood vessels. Three to 4 weeks after infection with a large amount of sporocysts (50,000 or more), the clinical signs include fever, anorexia, anemia, emaciation, and hair loss, mainly on the rump and tail in cattle, and some animals die. Pregnant animals may abort, and growth is impaired (Markus et al., 2004). Inoculation of pregnant does (75-105 d of gestation) with sporocysts of *Sarcocystis* resulted in abortion and neonatal death (Givens and Marley, 2008). Animals recover as cysts begin to mature (Dubey and Lindsay, 2006).

### ***Toxoplasma gondii***

Toxoplasmosis is a remarkably common parasitological disease caused by the protozoan *T. gondii*. The only known *Toxoplasma* species is *T. gondii*. Felids are the definitive hosts, and the other mammals and birds are intermediate hosts. The three infectious stages for all hosts are tachyzoites, bradyzoites (in tissue cysts), and sporozoites (Dubey and Lindsay, 2006). *T. gondii* is one of the main causes of infective abortion in New Zealand, Australia, UK, Norway, and the USA. Actual losses in lambs and kids due to toxoplasmosis are difficult to determine because the disease is usually sporadic; only a small number of aborted fetuses are submitted for diagnosis; those submitted may be inadequately examined; unsuitable material may be sent for diagnosis; the serologic test may not be specific; and toxoplasmosis does not cause clinical disease in the ewe/does, so this condition does not alarm the farmer as much as other bacterial and viral infections (Dubey, 2009).

Fetuses at all stages of development are susceptible to infection. Infection in early pregnancy usually results in fetal death and abortion, in which the fetus may be reabsorbed, macerated, mummified, or preserved. Infection at a later stage may result in a stillbirth or weak lamb/kid. Abortion levels may

vary from 5% to 100%. It is common to see mummies and stillborn kids or lambs within one litter, whereas other animals of the litter appear clinically healthy. Maternal immunity, timing of exposure and dose of oocysts determine the level of abortion in a flock or herd. Ewes and does infected before pregnancy will occasionally abort if reinfected. Flocks affected by toxoplasma abortion often have had contact with kittens, either directly or from fecal contamination of forages, water, or grain. Presence of mummies and changes at the placental cotyledons with small white foci of necrosis and calcification are suggestive of toxoplasmosis. Histological and immunohistochemical examinations will help to confirm the diagnosis. Polymerase chain reaction is generally effective (Innes et al., 2009; Menzies, 2011).

In flocks/herds in which toxoplasmosis was diagnosed, often little can be done during the lambing/kidding period. Control can be instituted either through feeding of prophylactic medications during pregnancy or by the use of a modified live vaccine. Feeding monensin at a dose of 16.8 mg per head daily reduces losses if delivered throughout gestation. Decoquinatate is licensed in the United Kingdom for the control of ovine abortion caused by toxoplasmosis. The drug is to be included in the gestating ewe ration at a rate of 2 mg per kg bw daily for the last 14 weeks of gestation; this is twice the recommended rate of inclusion to lambs and kids for the control of coccidiosis. Inclusion rates in the feed should be calculated to deliver this dose (Dubey, 2009; Menzies, 2011). A live vaccine (Toxovax1) is commercially marketed in the UK, France and New Zealand for reducing losses to the small ruminant production from congenital toxoplasmosis. This vaccine was originally developed in New Zealand, and additional efficacy studies were conducted in Scotland. The vaccine consists of a modified strain (S48) of *T. gondii*, originally isolated from an aborted lamb in New Zealand. By repeated passage in mice for many years, the strain lost the capacity to produce tissue cysts and oocysts. The commercial vaccine consists of living cell culture-grown tachyzoites that have a shelf life of 10 days. It is recommended to be given 3 weeks before mating. One subcutaneous injection of this 2 ml suspension induces protective immunity for at least 18 months (Buxton and Innes, 1995). It is also potentially zoonotic and must be handled carefully. However, the vaccine can be used in association to either a modified live chlamydial vaccine or an inactivated *Coxiella* vaccine, with no disruption of immune response or risk to the ewe/doe, a program that leads to animals being immunized against the two main causes of abortion (Menzies, 2011).

People handling meat should wash their hands with soap and water for preventing *T. gondii* infection. All cutting boards, sink tops, knives, and other

materials contacting uncooked meat should also be washed with soap and water. Washing is essential because the stages of *T. gondii* in meat are killed by contact with soap and water. *T. gondii* in meat is killed by exposure to extreme cold or heat. Cysts in meat are killed by heating to an internal temperature of 67°C or by cooling to -13°C. Meat of any animal should be cooked to a minimum of 67°C before eating and tasting meat while cooking or while seasoning. High-pressure treatment can destroy tissue cysts in meat and oocysts on fruits and vegetables. Salt additives to tenderize the meat can reduce inactivate tissue cysts in meat. Pregnant women should avoid contact with cats, cat litter, soil, and fresh meat. Cats should be fed only dry, canned, or cooked food, and the cat litter box should be emptied daily. Gloves should be worn while gardening and vegetables should be washed thoroughly before eating. People should avoid drinking unfiltered water from lakes, ponds, and rivers. Access to water reservoirs by cats should be prevented (Dubey and Lindsay, 2006; Dubey and Jones, 2008; Dubey, 2009; Menzies 2011).

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*Chapter 4*

**THE INFLUENCE OF ORAL ENVIRONMENT  
ON DIET CHOICES IN GOATS: A FOCUS  
ON SALIVA PROTEIN COMPOSITION**

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**ABSTRACT**

There is ample evidence that ruminants are capable of making choices between different foods that provide a more balanced diet that would be obtained by eating at random. In the particular case of goats, they occupy a diversity of habitats and different breeds present variability of feeding behaviors resultant from adaptations to the existent plant species. In their food search activity, individuals are faced with variable

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amounts of plant secondary metabolites (PSMs), which may present some toxic and anti-nutritional effects depending on the individual's ability to deal with it.

The oral cavity has a key role in the recognition and decision processes of ingestion or rejection. In this chapter we will first consider how goats identify foods and behave according to the food items available. Focus will be done on the importance of taste sense in this process and the information available on the main structures involved in taste detection and perception in goats will be reviewed. In a second section we will focus on the characteristics of goat's saliva, particularly in terms of their protein composition, presenting results obtained by our research team.

**Keywords:** goat, adaptive strategy, taste sensitivity, ingestive behavior, salivary proteome

## INTRODUCTION

Goats (*Capra hircus*) were domesticated around 7000 BC (Mason, 1981) and are present in different ecosystems but in higher number in the tropics, dry zones and developing countries (96% of the world goat population) (FAOSTAT, 2009). Goats are valued for the ability to exploit land of low productivity and marginal areas, as well as for their low cost and low labor management. This is an important husbandry species worldwide, which through time has been subjected to trait selection and breed differentiation (Galal, 2005). Actually 880 million goats, belonging to 570 different breeds are distributed worldwide (Dubeuf and Boyazoglu, 2009). This high biodiversity is concomitant with a high variability in feed availability and consequently results in different feeding strategies adopted.

Goats are characterized by their selective behavior. In normal conditions they graze or browse selectively, whereas in more stringent conditions of food availability they can be heavy browsers of trees and shrubs, and even consume foods that otherwise they would reject (Animut et al., 2005). Nitrogen content in vegetation plays a key role in nutrition and diet selection, since nitrogen is a limiting element for herbivores (Tipler et al., 2002). On the other hand, plant secondary metabolites, such as tannins, can be potentially toxic and/or anti-nutritive (being the intensity of the negative effects dependent on the defense mechanisms animals possess), resulting in avoidance of the plants presenting higher levels of these compounds (Provenza et al., 1992).

The ability to select and make the more suitable choices will depend on the capacity of goats to detect feed characteristics. The oral cavity has a key role in this process of recognition and in the decision of ingestion or rejection. On one hand, through taste receptor located in the mouth, animals may perceive some food characteristics. As such, the sensitivity for each class of taste is crucial. Basic tastes are usually associated to the level of particular food constituents and usually result in an innate response of acceptance or rejection. For example, sweet and umami taste appears to be characteristic of nutritious diets, being linked to the presence of carbohydrates and proteins, respectively. Inversely, bitter and sour tastes are associated to toxic or spoiled foods. On the other hand, saliva present in the oral cavity may also be determinant in dietary choices. This fluid characteristics influence oral medium and consequently may affect the way food is perceived. It may interact with food constituents altering their sensorial characteristics, both taste and mechanical sensations. One example of this last situation is the precipitation of polyphenolic compounds by particular salivary proteins, resulting in astringency perception.

Consequently, the particular characteristics of goats oral cavity, like in the other animal species, influence dietary habits and a comprehension of them may help to understand why these animals are able to select food that other species reject. In the present chapter we will focus on the oral characteristics of goats that may contribute to their characteristic feeding behavior, with focus on taste function and saliva. We will start for reviewing the characteristics of goats feeding behavior and after that the importance of both taste and saliva on this behavior will be critically discussed.

## **GRAZING BEHAVIOR AND DIETARY CHOICES**

According to Hofmann classification of ungulates goats belong to the intermediate feeding type. This means they will both graze and browse depending on food quality and availability (Hofmann, 1989).

Domestic goat is a specie greatly found in different ecosystems and whose feed strategies usually have an impact on vegetation (Papanastasis and Peter, 1998; Perevolotsky et al., 1998). However, in contrast to their negative image, concerning the effect on plant biodiversity, goats are also considered as useful biological agents of woody plant control (O'Connor, 1996), due to their particular dietary choices.

Although goats have a high capacity to adapt to diet conditions available [i.e. they depend heavily on plant availability (Barroso et al., 1995)], one of their ingestive characteristics is their very efficient selective behavior, picking some plants or plant parts, whereas others are totally or partially rejected. This allows these animals to improve the nutritive value of total ingesta. In general, they prefer nutritious food and avoid foods with low nutrient content or high levels of toxic/antinutritive compounds (Bryant et al., 1991; Provenza et al., 1992). Goats in temperate climates, where foraging availabilities are relatively equilibrated and nutritiously uniform, may behave similarly to other domestic ruminants (e.g. sheep and cattle), having no need of special selective skills for high-quality diet. However, in harsh environments, this specie has the ability to thrive better.

The influence of plant species available on goats diet selection pattern was observed in animals living in different environments, namely in zones of high plant diversity (Hendricks et al., 2002), Mediterranean arid scrublands (Barroso et al., 1995) or semi-arid savannas (Dziba et al., 2003). In harsh environments browse is clearly a major component of the diets (Pawełek et al., 2008). In such conditions, goats are able to utilize the scanty shrubby resources, selecting the more nutritive parts and converting them in a useful product. Browse species are more important for these ruminants production during dry season when herbaceous species have a poor quality and have limited biomass (Abdulrazak et al., 2000). The plant parts usually chose as feed include leaves, tender shoots or twigs, fruits, pods and seeds (Aganga and Tshwenyane, 2003).

The grazing characteristics of goats are different from other grazing ruminants not only in the type of plant and plant parts choose, but also in the way they bite. Whereas sheep show a tendency to penetrate into the canopy to take deep bites on legumes, goats appear to graze from the top downwards. Physically it might be because goats are less able to exert the force necessary to graze lower down into swards. Sheep usually had greater bite weight and larger bite volume than goats when grazing vegetative and reproductive legume swards (Animut and Goetsch, 2008). Goats showed a greater disposition to graze all accessible components of reproductive swards, especially reproductive grasses. However, the goat ingestion profile changes seasonally, according to the type of feed available. The highest bite rates appear to be achieved during the dry season, comparatively to wet season (Yayneshet et al., 2008). It was tentatively explained to result from the low availability of forage existent during the dry season, what could result in an increase the bite sizes.

Selection by goats between diverse plant species (Dziba et al., 2003) or between individuals of the same plant species (Riddle et al., 1996) is greatly performed according to plant nutritional quality or the concentration of chemical defenses. These animals feeding behavior adapts to food physical and chemical characteristics (du Toit et al., 1991; Provenza et al., 1992; Villalba and Provenza, 2000). One of the drawbacks of browse species is their relatively high content of defense mechanisms against herbivory. Among there are structural factors, such as the presence of morphological structures (e.g. spines, thorns and prickles), which limit the access to animals, and the fiber content (e.g. cellulose, hemicellulose and lignin). Although structural factors are not associated with animal intoxication, they reduce forage intake (Shipley et al., 1998), digestibility (Edwards and Ulrey, 1999), or both, and consequently animal performance is adversely influenced. One of the goats physical characteristics that allow them to select plants and plant parts, even with these structural defense characteristics, concerns their mobile lips and precise tongue movement. This makes possible to take only the fragments of interest, leaving the unchosen ones and allows the selection of nutritious materials even from low accessible sites (Illius et al., 1999).

Besides these structural defense mechanisms, plants (and principally browse) possess a wide variety of chemicals, which function as feeding deterrents, reducing forage value by being antinutitive and/or toxic and resulting in sickness and even deaths. These plant secondary metabolites (PSMs) have a negative impact on the fundamental biochemical processes (e.g. survival and growth) and on the selective behavior of herbivores. Despite the high number of existing PSMs (e.g. alkaloids, essences, terpenes), tannins constitute one of the most important groups. Tannins are mainly found in woody species and probably have the largest influence on the nutritive value of browse as forage (Reed, 1995). Tannins are commonly divided into two groups: hydrolysable and condensed tannins (Butler et al., 1999). The anti-nutritive value of browse is mainly attributed to condensed tannins (Reed, 1995). The presence of these PSMs is one of the principal conditioning factor of goat feed choices, although the nutritional fractions (e.g. protein, soluble carbohydrate, fiber) are also frequently connected with palatability (Malachek and Provenza, 1993).

It was observed that goats can tolerate a relatively high intake of tannins and can, therefore, feasibly increase their nutrient intake by ingesting plants with these PSMs for limited periods of time (Provenza et al., 1990). The capacity of ingesting a diet with higher levels of tannins than grazer species has been attributed to the presence of diverse defense mechanisms. For

example, the presence of tannin-resistant bacteria in goat rumen, which is capable of clearing tannin-protein complexes, was presented as one of these mechanisms (Brooker et al., 1994). Salivary proteins were also reported as a first line defense mechanism present in species for which regular diets are usually high in tannins. The binding of salivary proteins to plant chemical compounds modulates their oral perception, affecting taste and preferences. This issue will be further on detailed.

## TASTE FUNCTION

Taste reception takes place in taste cells, located on papillae, distributed on the dorsal surface of the tongue, soft palate, pharynx, and the upper part of the oesophagus (Lindemann, 2001). Despite the importance of these structures in taste, the sense of smell together with oral tactile sensations (texture of food, temperature and stimulation of pain endings), greatly alter the taste experience (Ginane et al., 2011). The importance of taste lies in the fact that it allows the selection of food based on its constituents, in accord with pleasure (hedonic factors) and with the body tissues' metabolic need for specific substances (homeostatic factors) (Salles et al., 2011). So, by being the sense involved when food are swallowed, taste is fundamental for animals regulating the intake of suitable foods and rejecting the unsuitable ones.

Taste buds are mainly located in papillae. The types, numbers and distribution of papillae in the tongue vary greatly among species. The lingual mucosa of goats, as for other domestic ruminants, exhibits differentiated types of papillae that can have gustatory and mechanical functions. In the tongue of goats, five types of papillae can be found: filiform, large conical, lenticular, fungiform and vallate (Kumar et al., 1998). Among these, fungiform and vallate papillae are the ones with taste perception functions.

The filiform papillae are conical-shaped, with 3-6 pointed projections and 6-8 secondary papillae at the free tip and the base of the dorsal surface of the tongue, respectively. These papillae have only a mechanical function. The large conical papillae have a round base and a blunt tip without any projection, and are found on the torus of the tongue. The lenticular papillae are present in close relation with the vallate papillae, having a wide range of sizes. In fact, two types of lenticular papillae can be distinguished, one with blunt apex and other, more frequent, with pointed apex and pyramidal shape (Kumar et al., 1998) and are characteristic of ruminants.



The fungiform papillae are smooth papillae, with a rounded surface, mainly located on the anterior and lateral anterior parts of the tongue. These papillae have a convex shape, raise above the lingual mucosa and are scattered among the filiform papillae, being smaller on the ventral surface than on the dorsal (Kumar et al., 1998). Their number is relatively high, but varying among different species. Even within species fungiform papillae number differs among different individuals. The vallate papillae are the largest tongue papillae and they are usually present in a small number. The vallate papillae are characterized by a papillary groove and an annular pad, and taste buds are present beneath the papillary epithelium. The number of vallate papillae differs among the several animal orders: a reduced number is observed in rodents and some omnivores, a slight increase in man and carnivores and a markedly higher number in herbivores (Table 1).

Like the other ruminants, goats tongue presents a lingual torus (lingual prominence). This appears to be a characteristic structure which has developed primarily in grass eating animals (Zheng and Kobayashi, 2006).

**Table 1. Differences in the number of vallate papilla among diff. Species**

Animal specie	Feeding type	Vallate papillae (number)	Reference
Cattle ( <i>Bos taurus</i> )	Herbivore Ruminant (grazer)	24-30 8-17	Davies et al., 1979 Agungpriyono et al., 1995
Sheep ( <i>Ovis aries</i> )	Herbivore Ruminant (grazer)	18-24	Agungpriyono et al., 1995
Goat ( <i>Capra hircus</i> )	Herbivore Ruminant (intermediate)	12-18	Agungpriyono et al., 1995
Lesser mouse deer ( <i>Tragulus javanicus</i> )	Herbivore Ruminant (browser)	2-5	Agungpriyono et al., 1995
Horse ( <i>Equus caballus</i> )	Herbivore Monogastric	2-3	Pfeiffer et al., 2000
Pig ( <i>Sus scrofa domestica</i> )	Omnivore	1-2	Montavon and Lindstrand, 1991
Man ( <i>Homo sapiens</i> )	Omnivore	12 7-9	Kobayashi et al., 1994 Jung et al., 2004
Dog ( <i>Canis lupus familiaris</i> )	Carnivore	4-6	Holland et al., 1989
Cat ( <i>Felis catus</i> )	Carnivore	7-8	Robinson and Winkles, 1990

Taste reception occurs in the taste receptor cells located in the taste buds. Taste stimuli reach the apical end of the taste receptor cell. This interaction

results in an afferent signal, which is transmitted to the central nervous system via three cranial nerves [chorda tympani (VII), glossopharyngeal nerve (IX) and vagus nerve (X)].

In terms of taste perception, goats are able to distinguish the five basic taste modalities - bitter, salt, sweet, sour and umami – through their lingual taste receptors (Ginane et al., 2011).

It is thought that taste sense has evolved to allow the recognition of food characteristics, ensuring the choice of a diet suited to body needs and the avoidance of toxic or antinutritive feed. Bitter and sour tastes are often associated to the presence of toxic and spoiled food, respectively. Sweet taste is present in carbohydrate rich foods. Salty taste is associated to the presence of sodium or salts in general. Concerning umami taste, the most common umami taste stimulus is the amino acid L-glutamate, and as such this taste is normally referred as indicating the presence of proteins.

It has been shown that taste perception differs according to animal species, which appears to be related to dietary needs. In herbivores in general, and consequently in ruminants, bitter taste has the particular importance of being associated to the presence of PSMs. Bitter taste receptors belong to the T2Rs superfamily of G protein-coupled receptors. There are diverse T2Rs genes coding for bitter taste receptors, which number is variable according to the animal species. Twelve functional genes were identified in cow versus thirty seven in rats (Shi and Zhang, 2006). This difference among species appears to relate to the variety of toxins usually found in the animal regular diet (Nei et al., 2008). It is possible that ruminants may have developed a low sensitivity (and consequently a high tolerance) for bitter compounds, since they need to accept some bitterness in order of not to limit too much the food ingested.

The number of studies about ruminant taste sensitivity is limited, and most derive from behavioral experiments (e.g. Robertson et al., 2006). In general it appears that the sensitivity of ruminants to the basic tastes is in the order bitter>sour>salty>sweet (Goatcher and Church, 1970). However, the taste thresholds (concentration levels of a tastant necessary for being perceived) appear to be lower in cattle than in goats and sheep. Apart from goats having lower taste thresholds than sheep, they can tolerate higher levels of tastants than sheep. These results may be discussed in function of the feeding habits of the different species (Goatcher and Church, 1970; Glendinning, 1994). Cattle and sheep are grazers, whereas goats may behave like grazers or browsers depending on plant availability. Goats may have a diet with high levels of browse, which frequently produce bitter-tasting compounds. If goats encounter bitterness more often than grazer species, it is possible that they are more able

to cope with this sensation through physiological mechanisms, among which saliva may have a primordial role.

## **SALIVARY GLANDS AND SALIVA PROTEOME**

Saliva is the liquid that bathes oral cavity, the local of food entrance and in which taste and aroma compounds are released. Moreover, saliva presents constituents that can interact with food components, influencing their perception (Spielman, 1990). As such, it has an important role in food perception and preferences.

Saliva has a major importance for diet adjustment as it serves as physiological buffer against variations between the animal external and internal milieus. It is produced by three pairs of major salivary glands (parotid, submandibular and sublingual) and numerous minor salivary glands, being this classification in “major” and “minor” based on the amount of saliva produced. Apart from the general characteristics, salivary glands are highly diversified structures exhibiting a complex degree of heterogeneity among the different animal species, both in location, development, microstructure and function (Phillips and Tandler, 1996). The vast multiplicity of diet chemical composition can contribute to diversity in salivary glands characteristics and saliva composition. A general conclusion that emerges from the comparative studies performed by Tandler and co-workers (Tandler et al., 1986; 1997; 1998; 2001) is that in mammalian species that have specialized diets, the major salivary glands exhibit differences when compared with relatives that are dietary generalists, presenting evidences that salivary glands are intimately related to dietary characteristics.

The normal composition of ruminant saliva is quite different from the saliva of monogastric animals: it is an isotonic bicarbonate phosphate buffer secreted in large quantities and with a high pH (8,2) (McDougall, 1948). Apart from the general functions described earlier for saliva, in goats, equally to ruminants in general, it has an additional major purpose of maintaining rumen homeostasis, by avoiding high and rapid drops of pH due to ruminal fermentation (McDougall, 1948). The high content of phosphates characteristic of ruminant saliva, besides providing alkalinity, is an additional phosphorus source for rumen bacteria (Breves et al., 1987). A large fraction of whole saliva (about 50-60%) is supplied by the parotid glands. The submandibular glands secrete only about one-eighth as much saliva as the parotid gland and most of this saliva is secreted during periods of feeding (Kay, 1960).

Ruminants are known to produce saliva with widely varying volumes and protein concentration, depending upon circumstances, such as if the animal is resting, eating or ruminating. This is due to the different contribution of each gland according to the conditions. Parotid saliva is maximal stimulated at the onset of eating but volume rapidly decline during meal (Carr and Titchen, 1978; Carter and Grovum, 1990; Meot et al., 1997). Eating effects on the parotid gland volume vary both according to the nature of diet consumed and the duration of a meal, inversely to what occurs for submandibular secretion. The amount of parotid saliva produced on a meal of fresh grass is higher than the one produced on a dry food meal, inversely to what occurs with submandibular saliva secretion, for which volumes are higher on dry foods (Carr and Titchen, 1978; Carr, 1984).

Similarly to other ruminants, the structure of parotid gland cells in goats is suggestive of copious secretion of saliva with a low protein concentration (Elewa et al., 2010). Parotid saliva concentrations of about 0,1 mg/mL were observed in these animals (Lamy et al., 2009). The total secretion of saliva per day, in these animals, has been estimated to be 6 to 16 liters (Elewa et al., 2010).

In previous points of this chapter the particularities of goat ingestion have been elucidated. There are a significant variability in goat breeds and habitats that also reflect variability in ingestive behavior and adaptations. Hofmann (1989) related salivary gland size, particularly parotid gland size, with ruminant feeding strategy. Accordingly, the ratio salivary glands weight (with more emphasis to parotid gland/total body weight) was thought to increase with the digestibility of the diet usually consumed, which means that concentrate selectors would have higher salivary glands weight than grazers. In this way, the size of the salivary glands would reflect a functional relationship between the mass of the glands and the composition of the diet. Based on this assumption, goats would be expected to present salivary glands sizes in the between of browsers and grazers.

One explanation for the different sizes in parotid glands is thought to be related to their function of detoxifying PSMs present in feed (Hofmann, 1989). As it was referred before, one of the most important classes of PSMs, in goats' nutrition, are tannins. Parotid glands are considered responsible for the synthesis and secretion of salivary proteins with a high affinity for tannins, being considered as a first line defense against the potential toxic and/or anti-nutritive effects produced by these PSMs. In general, it is greatly reported that animals feeding in a vegetation rich in tannins might develop the competence

of producing of such tannin-binding salivary proteins (TBSPs) (Shimada, 2006).

In ruminants it has been suggested the presence of such type of salivary proteins in concentrate selectors or browsers (e.g. deer) and their absence in grazers (e.g. sheep and cattle). Moreover, changes in salivary protein profiles have been observed to be induced by high levels of tannins in diets, even in animal species which do not present TBSPs constitutively in their saliva (Lamy et al., 2011). Since goats are intermediate feeders, whose diets may present considerable levels of tannins, the presence of such a salivary defense mechanism could be a possibility. However, this issue remains controversial.

The induction of TBSPs in response to diets high in tannins, which was observed for laboratory rodents (Mehansho et al., 1985; 1987), was also hypothesized for herbivores such as goats (Robbins et al., 1987). However, to our knowledge, an exact identification of such salivary proteins was not performed until now. The high ability to counteract the negative effects of PSMs in tropical tannin-rich plants, by goats, was suggested to be due to the presence of TBSPs in their saliva (Alonso-Diaz et al., 2009; Alonso-Diaz, 2010). It is hypothesized that the presence of such salivary proteins can modify the astringency and post-ingestive effects of tannin-rich plants. Also in favor of the presence of TBSPs, it was reported a relatively richness in proline (6.5%), glutamine (16.5%) and glycine (6.1%) of goat parotid saliva, and an increase in parotid saliva concentration when these animals fed a tannin-rich diet, comparatively to a diet with low levels of these PSMs (Silanikove et al., 1996). By analyzing salivary glands, Vaithyanathan et al. (2001) also suggested the presence of TBSPs for goats.

However, in some experiments the secretion of TBSPs was not observed. Distel and Provenza (1991) did not find the most well studied type of TBSPs (the Proline-Rich Proteins – PRPs) in goat saliva. Instead, these authors argue that goats can consume amounts of tannins relatively high due to the presence of other different defense mechanisms. Recently, using proteomic techniques, PRPs were also not identified in goat parotid saliva, neither constitutively (Lamy et al., 2008; Lamy et al., 2009), neither when feeding a tannin-enriched diet (Lamy et al., 2011). Coincidentally, Hanovice-Ziony et al. (2010) reported the absence of goat salivary proteins that directly bind tannins (either tannic acid or quebracho tannins).

Despite the heterogeneity in reports about salivary defense mechanisms against tannins in goats, changes in parotid salivary proteome induced by tannin ingestion were observed (Lamy et al., 2011), and as such the involvement of saliva in the consumption of tannins, by this specie, may not be

discarded. Consumption of quebracho tannins (condensed tannins) resulted in the increase in expression of both the protein cytoplasmic actin 1 and the protein annexin A1. We cannot assure that these proteins act as TBSPs, and in fact they may be only the consequence of an increased salivary gland function, induced by tannins. Nevertheless the role of these salivary proteins in goat tannin ingestion deserves further elucidative studies.

Apart from TBSPs, goat, like the other animal species, present a diversity of salivary proteins (Lamy et al., 2009), and their salivary proteome needs to be deeply studied. Many of the identified proteins are also present in other animal species, but their exact function in goat saliva, and their relation to food perception is not completely elucidated. One of the already referred characteristics of goats is that they seem not reject bitter foods as intensely as other species (Church and Goatcher, 1970). Annexin A1, identified in goat parotid saliva when consuming regular diet (Lamy et al., 2009), and increased after tannin consumption (Lamy et al., 2011) was reported to be increased in human saliva after stimulation with bitter taste (Neyraud et al., 2006). Although its role in bitter taste detection had not been mentioned, it should not be discarded a potential involvement in the bitter perception of tanniferous plants by goats.

Additionally to the mentioned salivary proteins, many others may be also involved in ingestive behavior and feed choice. For example, the salivary protein anhydrase carbonic VI [which is present in different isoforms in goat saliva (Lamy et al., 2009)], has been linked to taste sensitivity (Tatcher et al., 1998). Other salivary proteins are being studied for their involvement in food perception in humans (Dsamou et al., 2011), and it is to expect that also in animals salivary proteins can modulate food perception and condition feed preference.

In conclusion, saliva modulates the way feed is perceived. Further advances about goat saliva composition might increase the knowledge on how food is perceived by these animals. It is important to highlight that most of the divergence existing in goat saliva composition may be derived from the huge variety of breeds and habitat conditions existent. Goats which live in temperate climates may present considerable differences when compared with goats living in arid or tropical areas. These factors should be taken into consideration when conclusions about the involvement of saliva in goat food intake are to be taken.

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## CONCLUSION

Goats are small ruminants presenting a great biodiversity. Goats have increased very much in number not because they are more productive than other domestic ruminant species, but because they are adapted to very different conditions, allowing them to have good performances in a variety of habitats with a diversity of feed resources available. The adaptive potential of this specie results from the development of physiologic mechanisms. Among them the oral cavity has a major importance. First, through taste sensitivity and food perception, it is involved in plant selection and in the decision process of ingesting or not. Animals assign a signal value to taste, which allow them to distinguish between nutrients and antinutritive/toxic compounds. Moreover, saliva composition has a critical role in goat ingestive behavior, since it modulates feed sensorial characteristics, on one hand, and, in the other hand, some salivary proteins bind PSMs, namely tannins, impeding them to act negatively in digestive tract. In that way it would avoid post-ingestive negative effects that would result in conditioned feed avoidance.

Whereas taste function and salivary proteome studies have increased for humans and laboratory animals, studies investigating how goats perceive basic tastes and how salivary protein composition contributes to ingestion process are still few in number. Studies about goat oral cavity characteristics, namely taste function and salivary characteristics, might allow improving prediction of diet selection, and consequently improving goat production.

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*Chapter 5*

## **ACUTE PHASE PROTEINS AS BIOMARKERS OF MASTITIS IN DAIRY GOATS**

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### **ABSTRACT**

Mastitis is probably the most costly of the infectious, endemic diseases to affect dairy species. Though most mastitis occurs as a low-grade, subclinical infection, it increases milk leucocyte content, reduces milk production and increases milk bacterial content. These all contribute to reduced milk value as a food and in monetary terms. The prevalence of such infections is also a significant risk to uninfected animals in the herd. The detection level of subclinical infections within a herd is very poor and the currently available tools for diagnosis are of limited use. This is especially so in goats, which have very high normal somatic cell counts because of their apocrine secretory anatomy.

Lately, there has been a lot of excitement about the application of tests for the acute phase proteins (APPs) to monitor animal health. It has been shown that increases in concentration of APPs can be measured in milk of mastitic goats before the appearance of clinical signs. The combination of several APPs with different responses may be used to achieve the highest sensitivity. Moreover, use of these combined APP indices could be employed in monitoring recovery following antibiotic treatment. It is expected that in the near future the development of

cheaper automated assays for determination of major APPs and the increase in the number of experimental studies about APPs response will contribute to a wider use of these proteins as biomarkers of the disease. Therefore, their inclusion in the routine biochemical profiles in dairy goats and other species will be of great diagnostic value.

## INTRODUCTION

Mastitis is a major disease condition limiting milk production in dairy goats. It is a general term that refers to inflammation of the mammary gland regardless of the cause. Mastitis is usually recognized by clinical signs, most obviously by abnormalities in the milk and the udder. The disease is usually local but may become systemic, especially in cases where coliforms are involved [1]. Bacterial infection is the usual cause of mastitis with more than one hundred and thirty micro-organisms having been reported to cause the disease [2, 3]. *Staphylococcus aureus* is the most important mastitic pathogen of most goat herds [4, 1]. Other organisms including several species of *Streptococci* such as *S. agalactiae*, *S. uberis* and *S. dysagalactiae*, are commonly isolated from infected udders [1]. Additional organisms less commonly isolated from mastitic glands in goats include *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Pasteurella haemolytica*, *Corynebacterium pseudotuberculosis* and *Mycoplasma* species [1, 4, 5].

The number of animals infected with mastitis is underestimated in most farms. Clinical cases are easy to spot, but subclinical cases show no obvious signs and can only be determined from microbiological examination of milk or measurement of non visible milk abnormality. Subclinical cases are 15 to 40 times more prevalent than clinical cases [1], and because they largely go undetected, they are responsible for the greater losses and impact on the animals' health.

The most common method of diagnosis of mastitis involves the counting of somatic cells, mostly leucocytes, in the milk by means of an electronic particle counter [1, 5]. Because of the apocrine secretory process in goats, there is higher rate of epithelial cell sloughing and the presence of cytoplasmic masses in goat milk [1]. Electronic particle counters cannot differentiate between these cytoplasmic masses and somatic cells, and the differential staining process required to confirm such counts are too laborious and time consuming. Furthermore, Somatic cell counts vary with so many factors such as breed, age, lactation period, season, nutrition, milking technique and the health



condition of the udder [6, 7]. Animal-side test such as the California mastitis test (CMT) are available but laboursome and time consuming if applied to a large number of animals. The CMT reagent reacts with deoxyribonucleic acid (DNA) of somatic cells present in milk to form a gel. The appearance of the gel is used to gauge the amount of somatic cells in the milk. In addition to this test having the problems inherent in determining SCC in goats, it is estimative and subjective, and therefore not reliable in detecting borderline cases of subclinical mastitis [8]. Microbiological tests are also not reliable as even clinical mastitis can occur without positive microbiology.

Evidently, there is great need for the development of rapid, sensitive methods that can be incorporated into routine herd surveillance for mastitis, especially of the non-obvious subclinical form. Results from several caprine studies have suggested that the acute phase proteins may indeed have such a potential and emphasis should be placed on exploiting this.

## **THE ACUTE PHASE RESPONSE**

The acute phase response (APR) occurs shortly following infection, inflammation, trauma, burns, malignancies, various other forms of tissue damage, and even stress. During this response, the biosynthetic profile of the liver, and other tissues such as the pancreas and mammary epithelium, are radically altered to produce higher levels of certain proteins [9, 10, 11]. The magnitude and type of the acute phase response is dependent on the host and on the stimulating factor [12]. Bacterial infections and other infections where tissue invasion is involved are amongst the most potent stimulators [13, 14]. The purpose of the APR is to prevent further injury to the organ, to isolate and destroy the infective organism, to remove the harmful molecules and debris, and to activate the repair processes that are necessary to return the organ to its normal function [15]. The APR is later followed by the specific immune response, which in contrast is selective.

## **ACUTE PHASE PROTEINS AS BIOMARKERS**

Investigations have shown that the quantification of acute phase proteins (APP) concentration in body fluids can provide important diagnostic information in the detection, prognosis and monitoring of diseases [16, 14].

Assessment of the concentration of APPs provides a convenient means to estimate the combined effect of the pro-inflammatory cytokine stimulation of the APR [17, 18]. APR is mediated by release of proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha from macrophages and monocytes at the site of infection or inflammatory lesion [12, 40]. These cause further stimulation of cytokine release and eventual systemic release of the cytokines. The various organs such as the liver and mammary glands are then stimulated. For instance, IL-6 binds to its receptors and causes phosphorylation of the transcription factor, which is then translocated to the nucleus where it mediates the transcription of acute phase genes [17, 40]. The effect of these cytokine actions appear quite early in the inflammatory process and therefore APPs have proved useful for early detection of infections. Moreover, several studies have reported that APPs can be used as biomarkers to assess the efficacy of therapeutic treatments against several pathologies [19, 14]. Indeed, measurement of selected APPs has been found useful for monitoring success of treatment in various diseases including diseases in primates [14] and in dogs [15].

The APPs are of avid interest in present day research because of their potential as markers of disease. Indeed, various infections can be detected before the appearance of clinical signs by the measurement of APPs [20]. Moreover, APPs have proved, in certain cases, to be useful for determining the severity of tissue damage in infections [13, 14, 20].

Lately, emphasis has been given to studies that would lead to the application of tests for acute phase reactants to monitor animal health. Indeed, field studies have been done that have found certain APPs to be sensitive and specific tests for detection of „normal“ or healthy animals and good candidates for incorporation into quality systems to evaluate the health status of herds. Such APPs that have been found useful for field use include pig-MAP for porcines [21], Hp for bovids [22] and SAA for sheep [39].

## **ACUTE PHASE PROTEINS IN DETECTION OF MASTITIS**

Several authors have suggested the application of APPs as markers for the early diagnosis of mastitis [9, 23]. In bovids, it has been shown that the APPs are present in milk during clinical and sub-clinical mastitis, with haptoglobin and a mammary-associated amyloid A being identified during this disease [9, 27]. Furthermore, precolostrum mammary tissue [10] and mastitic mammary epithelium have been shown in bovids to form mammary serum amyloid A

(mSAA) [11]. However, the acute phase response is very specific and there may be great variations across species. This necessitates that each species be studied individually.

In goats, there appear to be varying reports from different investigators. Haptoglobin (Hp), serum amyloid A (SAA) and acid soluble glycoprotein (ASG) have been shown to be acute phase reactants following subcutaneous injection with turpentine [28], while C-reactive protein (CRP) is not [29]. The APPs have shown great potential as early markers in other goat diseases such as sarcoptic mange [30] and ruminal acidosis [31]. However, the potential of these APPs for use as biomarkers in goat mastitis is still largely unexplored and unexploited. Few investigators have ventured into this area and published reports appear quite varied. Winter *et al* [25] found that SAA cannot be used as a diagnostic tool for mastitis of mixed aetiology in goats, although *S. aureus* positive milk samples showed significantly higher SAA concentrations than those from healthy udders. Mungatana *et al* [32] found Hp and SAA to be markedly increased in local Kenyan goats experimentally infected with *S. aureus*, as early as 24 hours following infection. The study also found that Hp and SAA concentrations agreed with somatic cell counts, metabolic markers and microbiological findings of the studied mastitic and healthy samples. These findings demonstrate that the changes observed in levels of Hp and SAA were as a result of the infection. This was further proved when risen levels of Hp and SAA in milk and serum of infected does was observed to resolve following curative treatment. Additionally, these results showed the APPs to be good indicators of the healing process following treatment.

Studies so far conducted suggest that APPs can be measured in serum and in milk to detect risen levels as an indication of caprine mastitis, especially that of the elusive subclinical form. However, milk testing has an advantage in that samples are easily obtained in a way that is less stressful for both the animal and the farmer than obtaining a blood sample and it can conveniently be automated and used for routine monitoring of dairy herds. At the same time, each goat half is an individual anatomical unit. Unlike serum testing, milk testing allows for each half to be sampled separately. If APPs are produced locally in the udder as a response to mastitis, they might be more rapid and sensitive markers of acute inflammation than the somatic cell count and other traditional methods.

## CONCLUSION

It appears that caprine mastitis can be detected before the appearance of clinical signs by the measurement of APPs. The combination of several APPS with different responses may be used to achieve the highest sensitivity. Various authors have proposed calculation of an index from rapid and slow acting APPs to enhance sensitivity and specificity in diagnosis [15, 11, 33]. With reference to normal levels of various positive and negative acute phase proteins, an acute phase index (API) may be calculated to increase the sensitivity of non-healthy condition assessment. A good example of this has been done for poultry by including SAA, transferrin, serum albumin, apolipoproteinA-1 [33]. In veterinary medicine, the API has been designed for bovids [11, 34], where quick and slow positive reacting proteins are combined with quick and slow negative reacting proteins. As soon as the most suitable proteins are defined for caprine species, this tool can be applied as an indicator for health, non-health and to define recovery from periods of illness.

There is great need for the development of automated technology to quantitatively measure APPs in a way that is easy and time-saving. Such technology would be a useful incorporation into quality systems for the routine evaluation of the health status of animal herds. The screening would then allow for specific diagnostic methods and therapeutic intervention to follow only if necessary.

Several scientists have developed such robust technologies that allow for the easy, routine measurement of APPs. Åkerstedt *et al* [35] developed a rapid biosensor method for the determination of haptoglobin in bovine milk. New sensitive assays have also been specifically developed for canine Hp and CRP based on time-resolved fluorometry. This technique has allowed easy measurement of these proteins in various canine specimens [15]. A commercially available multi-analyte immunoassay for murine immunoassay profile has also recently been developed [36, 37]. It provides rapid and easy detection of 60 acute phase reactants, cytokines, chemokines, growth factors and hormones. A more recent development is the coupling of multi-analyses technology with pattern recognition software [38], which greatly improves the power of selective diagnostics of the APPs. However, it should be remembered that national and international standardization of the APP assays is required before they can be applied for the routine health monitoring in veterinary medicine.

Further studies should be designed in order to elucidate some unclear aspects of caprine APP's biology and pathology. These aspects include the

possible expression of APPs on circulating cells or the characterization of APP receptors. There is also need to investigate other APPs in caprine mastitis (both positive and negative) in order to elucidate possible interactions and develop an API for the species. Moreover, interpretation of the analytical data must be knowledge-based. Future possibilities for the application of APPs therefore depend on basic new mechanistic findings of known proteins, new discoveries such as organ-specific components and on technological possibilities for rapid chemical multianalyses with computer analyses of the patterns found.

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*Chapter 6*

**INFLUENCE OF FEEDING GOATS WITH  
THYME AND ROSEMARY EXTRACTS ON THE  
PHYSICOCHEMICAL AND SENSORY QUALITY  
OF CHEESE AND PASTEURIZED MILK**

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**ABSTRACT**

The use of natural antioxidants and flavenoids from by-products derived from aromatic plants can be considered as an alternative to using synthetic antioxidants in the food and pharmaceutical industries. In this sense, feeding goats with aromatic plant byproducts constitutes an interesting option for goat husbandry which increase the quality of the final products while reducing feeding costs. In this chapter feeding goats with *Thymus zygis* spp. and *Rosmarinus officinalis* spp. by-products was studied to determine the influence on the physicochemical composition

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(dry matter, fat and lactose content), cryoscopic point, somatic cells count of pasteurized goat milk, and on the physicochemical composition, microbiology and sensory analysis of Murcia al Vino cheese as a goat-derived product. In milk significant differences were observed in dry matter, protein and lactose contents while no significant differences were showed in fat, somatic cell count or cryoscopic point. In Murcia al Vino goat cheese no significant differences were determined in any of the physicochemical and microbiological parameters, although significant differences were observed after the sensory analysis, mainly in texture, taste and overall acceptance. The cheese produced with goat milk supplemented with *Thymus zygis* spp by-products showed higher overall acceptance. Both products can be considered of interest for contributing to the range of healthy foods increasingly demanded by consumers.

**Keywords:** *Rosmarinus officinalis* spp, *Thymus zygis* spp, Murcia al Vino Cheese, pasteurized milk, supplementation

## INTRODUCTION

Goat production constitutes an important part of the national economy in many countries, especially in the Mediterranean region and the Middle East. Such production is of particularly high importance in France, Italy, Spain and Greece. That goat milk is an excellent food source is undeniable. Goat milk has proven beneficial effects on the body's, physiological functions, and is ideal for the nutrition of children and elderly people. As has reported by several authors, goat milk can be consumed as an alternative to cow milk in people with allergies (El-Agamy, 2006). This type of milk has an acceptable, attractive odour and taste (Park et al., 2007).

The province of Murcia is characterized for its high annual Murciano-Granadina milk production and its facilities for the production of different cheese varieties (López et al., 1999). This goat breed is considered as one the most important breeds in Spain, both in number and milk production (Vacas, 2003).

Goat milk quality can be defined as the potential to tolerate technological treatments in order to become a product with the ability to satisfy consumers' demands, in health terms, nutritional values, safety and pleasure (sensory attributes). Cheese quality is closely related with milk composition and quality (Ribeiro and Ribeiro, 2010), while cheese yield is mainly related with milk fat and protein concentrations (Pulina et al., 2006). Milk quality can be evaluated

according to sanitary, dietetic, nutritional and technological criteria and cheese quality by also considering different physicochemical and sensory parameters. All these criteria depend on numerous factors and their interaction, which are linked to the main physicochemical components (fat, protein, lactose) of milk, as well as to the micro-compounds commonly found therein such as minerals, vitamins, short fatty acids, cholesterol and terpenes (Morand Fehr., 2007).

Cheese sensory quality refers to the cheese properties that are perceived by the senses. In sensory analysis, the consumer constitutes the best measurement instrument, and although obviously it is a subjective evaluation, if a suitable methodology is followed by trained tasters, the results of sensory analysis can be regarded as an objective method (Chamorro and Losada, 2002). Therefore, the measurement of sensory characteristics by tasteres is an important point for the producer. Cheese flavour is a critical parameter for marketing and is evaluated by graders who use a limited vocabulary of common flavour and texture defects to generate a quality grade. Descriptive sensory analysis is an analytical sensory technique which generates a specific flavor vocabulary and whose application to the product by a trained panel makes it a reliable (Drake et al., 2001).

Cheeses are identified by their specific sensory attributes, which are widely appreciated by consumers (Barcenas et al., 2001). In addition, the traditional character of a cheese and its designation of origin are two of the most important factors influencing consumer preference in the market (Bertozzi and Panari., 1993).

The importance of aromatic plants as natural antioxidants has been well described by many authors (Cuvelier et al., 1996) due to the presence of their principal secondary metabolites, polyphenols, which are characterized by having redox properties that allow them to act as reducing agents. The antioxidant properties of sage, rosemary and thyme, widely used in the Mediterranean area, have been studied previously (Parejo et al., 2002). Rosemary (*Rosmarinus officinalis*), one of the many aromatics plants currently exploited in the province of Murcia, generates an excess of residues after the leaf distillation for the manufacture of essential oils (Nieto et al., 2010). The natural polyphenols found in the leaves of rosemary have potential therapeutic benefits related to the high antioxidant activity of rosemary and its anticarcinogenic and antiviral properties, which have been observed by both in vitro and human assays (Aruoma et al., 1996). Jordan et al. (2010) describe how the introduction of distilled rosemary leaves into the diet of goats results in an increase in flavonoids (hesperidin, naringin, and genkwanin, galic acid and carnosol) in the resulting Murciano-Granadina milk.

*Thymus zygis*, also known as red thyme, is one of the most commercially valuable Spanish thymes due to the importance that the presence of thymol has for thyme essential oil quality. For this species, the average yield of essential oil per plant is around 3% (w/v) (Sotomayor, 1998). Currently, in Spain, more than 1500 tonnes/year of dry leaves and 26 tonnes of thyme essential oil are exported to foreign countries (Sotomayor, 1998). Different extracts from thyme leaves have shown the presence of a large number of flavonoids and vitamin E, compounds that are of great interest in the food industry due to their antioxidant properties (Guillén and Manzanos, 1998). The essential phenolic compounds on *Thymus zygis* are carvacrol methyl ether, thymol, carvacrol, eugenol (Jordan et al., 2009). The introduction of aromatic plants with a high level of polyphenol compounds, and their effect on milk composition (fat, protein, lactose, etc) and yield has not been investigated, although it has been reported that feeding cattle with a gossypol-rich diet can increase the yield, fat and non-casein nitrogen milk content (O'Connell and Fox, 2001).

Although, synthetic antioxidants have long been used in foods, their use is increasingly discredited due to their suspected carcinogenic potential (Chen, Shi, and Ho, 1992) and the general rejection of synthetic food additives on the part of consumers. A general swing towards the use of natural compounds has stimulated research into their use as antioxidant replacements. Several studies related with animals feeding and its influence on the milk composition have been carried out (Sanz Sampelayo et al., 2007), but there are few articles on the feeding of goats with by polyphenolic-rich aromatic plants and the effects on milk and cheese properties.

The aim of this chapter is to evaluate the influence of feeding goats with the by-products of *Thymus zygis* spp. and *Rosmarinus officinalis* spp. on the physicochemical composition (fat and lactose content), cryoscopic point and somatic cell count of pasteurized goat milk, and on the physicochemical composition of Murcia al Vino cheese.

To this end, thirty-six multiparous lactating Murciano-Granadina goats were fed *Rosmarinus officinalis* spp and *Thymus zygis* for sixteen months. A control group of goats fed without supplementation. Milk samples were collected every week and identified by codes for sixteen months. Two 50 mL samples were taken from each of the animals chosen and analyzed to determine the above mentioned physicochemical parameters. During this 16 month period, Murcia al Vino cheese was manufactured with the supplemented and control milk. This type of cheese is a washed-curd, non-cooked, semi-hard pressed cheese which is ripening for 45 days. Cylindrical in shape,

it has a smooth light rind that is bathed in red wine. Cheesemaking was carried out with the collaboration of an accredited expert cheesemaker and under the supervision of a representative member of the Board of P. D. O. Murcia al Vino cheese to ensure the manufacturing process followed the standard procedure for Murcia al Vino cheese. Henceforth, the milk and cheese obtained from goats fed a rosemary (R) and thyme (T) supplemented diet will be referred to as R-milk/cheese and T-milk/cheese, respectively.

Significant differences ( $P < 0.05$ ) were observed in the dry matter, protein and lactose content, between the control and supplemented goat milk (Table 1).

Contrast analysis pointed to significant differences between milk obtained from goats fed a rosemary or thyme supplemented diet (R and T, respectively) in all the parameters analysed except for the cryoscopic point. Supplementation of the goat diet with rosemary and thyme was seen to provide milk with a higher dry matter content. Rosemary supplementation increased the protein value while thyme supplementation led to a lower value.

**Table 1. Physicochemical and somatic cells of goat milk**

Type of feed	Dry matter (%)	Fat (%)	Protein (%)	Somatic cells count *( $\text{ml}^{-1}$ )	Cryoscopic point ( $^{\circ}\text{C}$ )	Lactose (%)
C	14.31	5.45	3.56	2530.8	-0.560	4.69
R	14.32	5.53	3.59	3250.8	-0.557	4.51
T	13.66	5.14	3.27	1488.3	-0.551	4.74
SEM	0.14	0.12	0.04	394.07	0.97	0.04
S. Level	**	NS	***	NS	NS	***
Contrast SEC						
C vs R	0.18 <sup>NS</sup>	0.14 <sup>NS</sup>	0.06 <sup>NS</sup>	491.71 <sup>NS</sup>	1.37 <sup>NS</sup>	0.00 <sup>NS</sup>
C vs T	0.21 <sup>*</sup>	0.17 <sup>NS</sup>	0.07 <sup>**</sup>	576.12 <sup>NS</sup>	1.37 <sup>NS</sup>	0.00 <sup>***</sup>
R vs T	0.23 <sup>**</sup>	0.19 <sup>*</sup>	0.07 <sup>**</sup>	629.43 <sup>**</sup>	1.39 <sup>NS</sup>	0.00 <sup>**</sup>

SEM: Standard Error of Means. S. Level: Significance level \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; NS: Non Significant, SEC: Standard Error of Contrast, C: control, R: *Rosmarinus officinalis* spp, T: *Thymus zygis* spp.

The lactose content decreased in the R- milk and increased in the thyme supplemented milk. Significant differences were observed between the control and the T- milk mainly in dry matter, protein and lactose content. As no differences were observed between the control and R-milk, feeding goats with

rosemary residues can be discarded as a potential method for modifying milk physicochemical features.

The effects observed of supplementation on the protein, lactose and fat content of the resulting milk disagree with the results published by Savoini et al. (2003) who introduced rosemary extract in Saanen goats' diet, while the result observed for the somatic cell count agrees with their findings. In milk from the Valle del Belice ewes consuming a diet supplemented with rosemary extract Chiofalo et al. (2011) determined an increased protein and lactose content, a decreased content and no significant difference in the somatic cell count. This agrees with our results for protein after rosemary supplementation and for lactose and somatic cell count for thyme supplementation.

The increase observed in the protein content both R and T- milk diets may be related with an interaction between the protein and the phenolic compounds (PC) in the reticulum, so that the microorganism in the rumen cannot act on the protein. However, once the PC-protein complex passes into the abomasum, the complex breaks down and the protein is degraded and used by the ruminant (O'Connell and Fox, 2001).

**Table 2. Physicochemical composition of Murcia al Vino cheese**

Type of feed	Dry matter (%)	Fat (%)	Protein (%)	Cheese yield (L of milk/Kg of cheese)
C	65.74	35.14	23.99	6.83±0.05
R	64.90	32.65	26.25	6.90±0.03
T	65.10	32.62	27.26	7.94±0.02
SEM	1.51	2.49	1.01	0.52
S. Level	NS	NS	NS	NS
Contrast SEC				
C vs R	1.92 <sup>NS</sup>	3.16 <sup>NS</sup>	1.28 <sup>NS</sup>	0.08 <sup>NS</sup>
C vs T	2.20 <sup>NS</sup>	3.62 <sup>NS</sup>	1.46 <sup>NS</sup>	0.08 <sup>NS</sup>
R vs T	2.40 <sup>NS</sup>	3.95 <sup>NS</sup>	1.60 <sup>NS</sup>	0.08*

SEM: Standard Error of Means. S. Level: Significance level \*\*\* P<0.001; \*\* P<0.01; NS: Non Significant, SEC: Standard Error of Contrast C: control, R: *Rosmarinus officinalis* spp, T: *Thymus zygis* spp.



**Table 3. Cheese Microbiology from Rosemary and Thyme supplemented milk (log ufc g<sup>-1</sup>)**

Type of feed (n=6)	Aerobics	<i>Enterobacter</i>	<i>S. aureus</i>	Molds, Yeast	Sulphite reducing	<i>E coli</i>	<i>Salmonella spp</i>	<i>Listeria spp.</i>
C	6.84	1.62	0.37±0.08	1.08	0	0	0	0
R	6.40	1.30	0	2.23	0	0	0	0
T	7.86	1.54	0	0	0	0	0	0
SEM	0.36	0.78	0.23	1.29	0	0	0	0
S.Level	NS	NS	NS	NS	NS	NS	NS	NS
Contrast SEC								
C vs R	0.48 <sup>NS</sup>	1.07 <sup>NS</sup>	0.31 <sup>NS</sup>	1.17 <sup>NS</sup>	0	0	0	0
C vs T	0.48 <sup>NS</sup>	1.07 <sup>NS</sup>	0.31 <sup>NS</sup>	1.17 <sup>NS</sup>	0	0	0	0
R vs T	0.56 <sup>*</sup>	1.23 <sup>NS</sup>	0	1.36 <sup>NS</sup>	0	0	0	0

SEM: Standard Error of Means. S. Level: Significance level \*\*\* P<0.001; \*\* P<0.01; NS: Non Significant, SEC: Standard Error of Contrast C: control, R: *Rosmarinus officinalis* spp, T: *Thymus zygis* spp.

**Table 4. Sensory parameters of cheese from Rosemary and Thyme supplemented milk**

Type of feed	Rind	Shape	Colour	Eyes	Texture	Odour	Taste	Residual taste	Overall acceptance
C	14.15	14.69	7.58	7.42	20.08	14.69	18.80	12.61	110.04
R	13.57	13.57	6.71	7.07	19.07	13.14	16.71	11.28	101.14
T	15.00	15.66	7.33	7.91	23.75	14.00	21.50	13.50	118.67
SEM	0.44	0.55	0.26	0.30	0.97	0.54	0.92	0.61	2.61
S. Level	NS	NS	NS	NS	**	NS	*	NS	***
Contrasts SEC									
C vs R	0.59 <sup>NS</sup>	0.73 <sup>NS</sup>	0.35*	0.40 <sup>NS</sup>	1.28 <sup>NS</sup>	0.71*	1.22 <sup>NS</sup>	0.80 <sup>NS</sup>	3.45*
C vs T	0.62 <sup>NS</sup>	0.77 <sup>NS</sup>	0.37 <sup>NS</sup>	0.42 <sup>NS</sup>	1.35**	0.75 <sup>NS</sup>	1.28*	0.85 <sup>NS</sup>	3.63*
R vs T	0.70*	0.87*	0.42 <sup>NS</sup>	0.48 <sup>NS</sup>	1.52**	0.85 <sup>NS</sup>	1.45**	0.96*	4.10***

SEM: Standard Error of Means. S. Level: Significance level \*\*\* P<0.001; \*\* P<0.01; NS: Non Significant, SEC: Standard Error of Contrast C: control, R: *Rosmarinus officinalis* spp, T: *iThymus zygis* spp.

Somatic cell counts are widely used to monitor of udder health and milk quality in the dairy industry of many countries, to assure quality milk supply from dairy farmers to consumers of milk and dairy products (Harmon, 1994). The maximum level in goat milk is limited to 1 million.ml<sup>-1</sup>, at least in the USA (Haenlein, 2002). In this chapter the values determined for the three lots of milk, provided values of 2530.8; 3250.8 and 1488.3 for control, R and T, successively, which are lowers than the values limited by permitted European legislation for goat milk. This result confirms the good sanitary condition of the milk (Todaro and Scatassa, 2001).

Regarding the physicochemical characteristics of Murcia al Vino cheese manufactured with the supplemented and control milks (Table 2) no significant differences ( $P>0.05$ ) were observed in any of the physicochemical parameters, even when a contrast analysis was applied. A slight difference in cheese yield was observed between rosemary and thymus supplementations. Indeed, the results highlight the potential of thyme supplementation to increase cheese yield, which is of great importance for cheesemakers.

As can be observed in Table 3, no significant differences ( $P>0.05$ ) were determined in cheese microbiology between either supplementation and the control, indicating that the administration of rosemary or thyme extracts in the goats' diet does not affect the microbiological quality of cheeses.

The sensory analysis pointed to significant differences ( $P<0.05$ ) in texture, taste, overall acceptance between R and T-cheeses and the control, as shown in Table 4.

The contrast analysis showed significant differences in colour, odour and overall acceptance between the control and R-cheese, in texture, taste and overall acceptance between the control and thymus supplementation, and finally in rind, shape, texture, taste and residual taste and overall acceptance between the rosemary and thymus supplementation. The highest values of rind and shape were observed in cheeses supplemented with thyme. Cheeses made with R-milk showed less whiteness than the control and the other R-cheeses. The supplementation of goats' diet with thyme did not affect cheese colour.

As regards the other sensory parameters, cheeses made with T-milk provided a better cheese texture. For odour, significant differences were observed between control and the R-cheeses, the lower values being observed in the supplemented cheese. No significant differences were observed in odour between the control and thyme supplemented cheese. This confirms that the odour from cheese made with R-milk was not acceptable to consumers. Significant differences were determined for taste between the cheeses. Cheeses from thyme supplemented milk showed higher taste values than those from

rosemary supplementation. The trained panel determined no significant differences for residual taste, although, contrast analysis provided to differences between both supplementations. The overall acceptance values determined by the expert panelists, confirmed that the cheeses made from the thyme supplemented milk are the most appreciated.

## CONCLUSION

The administration of rosemary leaves to Murciano-Granadina goats increases the protein values of the milk, while the supplementation of diet with thyme increases dry matter and lactose contents. Supplementations with rosemary and thyme did not affect the physicochemical and microbiological parameters of the cheeses produced. Supplementation with rosemary extracts did not affect the sensory attributes perceived by the panellists except cheese colour, odour and overall acceptance. Thyme supplementation provided better sensory attributes, demonstrating its greater potential for use in goats' diet supplementation.

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