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RVRALIS ET SILVESTRIS SAPIENTIA

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Ciclo XXIX

Modulation of dietary energy partitioning between milk production and body reserves in sheep and goats

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TABLES OF CONTENTS

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

GENERAL ABSTRACT

This thesis is a contribution in the definition of the optimal neutral detergent fiber (NDF) and starch levels in the diet supplied during the lactation of dairy goats and sheep, and to the comprehension of the mechanism involved in their utilization for milk production or body reserves accumulation. Based on the literature, it has been observed that the effects of dietary NDF and starch can vary with stage of lactation and small ruminant species. In general, goats seem to benefit from diets rich in starch throughout lactation, whereas in sheep starch seems to have a positive effect in early lactation but a negative effect in mid-lactation, when NFC favor body reserves accumulation and only digestible NDF favors milk persistency. It has been hypothesized that these differences could be linked to differences in the hormonal status that drives nutrient partitioning toward mammary gland or body reserves. Thus, a comparison between sheep and goats was needed to elucidate this mechanism, comparing these species in the same feeding, management and environmental conditions.

This thesis is organized in 4 main chapters.

Chapter 1. This introduction section reports a literature review on the classification of carbohydrates, on their productive effects and on the mechanisms affecting their partitioning during the lactation, with specific emphasis on dairy small ruminants.

Chapter 2. This chapter describes the productive results of an experiment carried out to study the effect of high-starch (HS) and low-starch (LS) diets in mid-lactation on sheep and goats simultaneously. The two species responded differently to the carbohydrates of the diet. In goats, the HS diet had a positive effect on milk production compared to LS diet. In sheep, the HS diet increased body fatness, whereas the LS diet had a positive effect on milk persistency.

Chapter 3. This part evaluated the hormonal and metabolic profile of sheep and goats from early to mid-lactation. The two species had a different hormonal and metabolic profile during the whole period studied. The higher growth hormone (GH) and lower insulin blood concentration observed in goats evidenced a better aptitude of this species to milk production compared to ewes. The lower GH and higher insulin blood concentration in sheep indicated that this species is more prone to body fat accumulation. The dietary treatments (comparison of HS vs. LS diets) had no effects on the hormonal and metabolic profile of the two species.

Chapter 4. This section measured the in *vivo* digestibility trials, using sheep and goats simultaneously, of a high-starch diet in early lactation and of high- and low-starch diets in mid-lactation. In addition, rumen pH, ammonia and volatile fatty acids were measured, together with the N balance and the microbial intestinal supply. The results showed that DMI, total energy requirements, ammonia concentration and microbial protein supply were higher in goats than ewes, in both stages of lactation. In mid-lactation, DM and starch apparent digestibility, and also TDN were higher with the HS than the LS diet, confirming the better aptitude to digestion of the HS diet, whereas NDF true digestibility was higher with the LS diet, likely due to the high content of digestible fiber present in this diet. Overall, it appeared that these factors did not vary enough to explain the productive differences observed in the research.

Finally, the dissertation ends with a summary of the main findings, a general discussion and final conclusions.

CHAPTER 1

Review of the literature

1. INTRODUCTION

Appropriate nutrition during the lactation of sheep and goats requires accurate dietary formulation and monitoring. However, while there is a vast research on energy and protein requirements of the ewes and the goats during lactation, with even some nutritional models based on the structure of the most advanced models for dairy cattle (e.g. Cannas et al., 2004; Tedeschi et al., 2010), unfortunately none of the existing feeding systems for small ruminants reports reference values for starch, non-fiber carbohydrates (**NFC**) and NDF in the diets used during the lactation of small ruminants. In contrast, many feeding systems for dairy cattle provide detailed guidelines for optimal NDF and NFC concentrations during the lactation (e.g. NRC, 2001). For this reason, this review will investigate the current knowledge on carbohydrates utilization in dairy sheep and goats.

1.1 Carbohydrates

Carbohydrates are organic compounds formed by carbon, hydrogen and oxygen in a ratio of 1:2:1 (Lehninger et al., 1994) that constitute 50-80% of the DM of forages and cereals (Van Soest, 1994).

As reported by Lehninger et al. (1994), they can be defined as key elements and energetic sources that support organism's life and are formed through photosynthetic process.

The photosynthetic process is the most important energy process that occurs in the world and that produces carbohydrates from inorganic compounds and light energy (Whitmarsh and Govindjee, 1999); the partitioning of these photosynthetic compounds into plant tissues determines the nutritive value of plants (Van Soest, 1994).

From a chemical point of view, carbohydrates are aldehydes or ketones linked to OH-groups and can be divided in monosaccharides, oligosaccharides or polysaccharides (Van Soest, 1994).

Monosaccharides are the monomeric unit of which the polysaccharides are composed. The typical linkage of carbohydrates is the glycosidic linkage, that permits the connection between two sugars or between a sugar and other molecules. Then through this type of linkage, many monosaccharides can link to form oligosaccharides (until 9 units), or polysaccharides (from 10 units) (Lehninger et al., 1994).

The most abundant carbohydrates in nature are polysaccharides such as starch, that is a reserve polysaccharide of plants cell, and cellulose, that is a structural polysaccharide of cell wall and that is the most abundant organic compound in the vegetable kingdom (Lehninger et al., 1994).

1.1.1 Classification of carbohydrates in animal nutrition

From the nutritional point of view, plant carbohydrates can be divided in simple sugars, reserves carbohydrates and structural carbohydrates (Van Soest, 1994).

Simple sugars and reserves carbohydrates are the most important energetic sources accumulated by the plants, thanks to the photosynthetic process. They include compounds accumulated in the cellular cytoplasm, such as simple sugars (glucose, fructose, oligosaccharides), and polysaccharides, such as fructans, and starch (Figure 1). A reserve function is also played by certain polysaccharides accumulated in the cell wall of certain plant species, such as β-glucans and galactans (Figure 1). All these compounds are used to sustain the growth and respiration of the plants; in perennial species they help the plant to survive to winter conditions, whereas during the spring they are important to favors their growth and regrowth after their use (White, 1973).

Structural carbohydrates (**SC**) are those that are present in the plant cell wall and that are irreversibly polymerized by the plant. They have a mechanical function, sustain the plants and protect them by external environmental factors and predators. They are composed by cellulose, hemicellulose, and pectins (Van Soest, 1994). The SC are closely associated and linked to non-carbohydrates compounds such as lignin and cutin, and, in certain grass species, by silica. In addition, certain proteins are strongly associated to the cell wall of the plant and should be considered a part of it. All SC but not pectins make the plant insoluble fiber and are quantitatively recovered in the NDF fraction (Figure 1). The insoluble fiber, quantified by NDF, can be divided in two

nutritional subfractions: degradable NDF, undegradable NDF. The first fraction represents the portion of NDF that is potentially fermentable (**pdNDF**) at rumen or lower tract level. It is composed by the cellulose and hemicellulose not encrusted by lignin or tightly bound to silica or Maillard products. The degradation rates of pdNDF vary depending on the feed considered and on its physical form, while the extend of degradation is inversely proportional to the rumen passage rate of the feed in which the pdNDF is included (Van Soest, 1994). Certain feedstuffs have a very high proportion of NDF in the form of pdNDF and have very high degradation rate of this fraction. Thus, they are very rich in digestible fiber. Among them, besides immature grasses and legumes, there are some by-products, such as soyhulls, beet pulps and citrus pulps that have a total NDF digestibility higher than 80%. They are also very rich in soluble fiber. The undegradable fraction of NDF (**iNDF**) is made by all fractions that cannot be fermented, regardless the retention time in the rumen. The iNDF of the plants is made by lignin, silica, cutine and by the cellulose and hemicellulose encrusted by lignin. The iNDF can be quantified after very long fermentation times (>240 h) with in situ or in vitro assessments or can be estimated by using mathematical coefficients or functions (Van Soest, 1994). For example, the CNCPS (Fox et al., 2004) and the Small Ruminant System (Cannas et al., 2004; Tedeschi et al., 2010) assume that iNDF is equal to ADL x 2.4.

Pectins, that have a structural function, and β-glucans and galactans, that have a reserve function even though part of the fiber of the plant, are often measured together and compose the so called soluble fiber, which is recovered in the neutral detergent solubles, together with simple sugars and starch, in the NDF analytical method (Van Soest et al., 1991) (Figure 1).

As said before, the sum of simple sugars, starch and soluble fiber defines the so called NFC, usually calculated by difference as: 100 - Crude protein – NDF – Ash - Fat. Pectins have been ascribed to NFC, even though they are a component of cell wall, because nutritionally similar for their solubility and rumen degradability, to reserve carbohydrates. The sum of sugars and starch determined analytically defines instead the so called “non-structural carbohydrates” (**NSC**).

1.1.2 Ruminal metabolism of carbohydrates

Carbohydrates have a fundamental role in ruminant nutrition because they produce 70-80% of their total caloric requirements. In ruminants, most carbohydrates are fermented in the rumen and lower tract, but part of them can escape the rumen and be digested in the small intestine, once cleaved in their monomeric forms (Owen and Goetsch, 1988).

Fermentation is a process that occurs in anaerobic condition and permits to the hydrolysis of the polysaccharides. In terms of efficiency, fermentation is less efficient than respiration because produces only from 2 to 5 ATP molecules, whereas in the respiration process produces 38 ATP molecules. Then, to produce the same amount of energy, the fermentation process needs to ferment a much larger amount of substrate.

As reported by Owen and Goetsch (1988), fermentation that occurs in the rumen is the result of physical and microbiological activity.

With the fermentation of carbohydrates, microorganisms produce volatile fatty acids (**VFA**), CO_2, CH_4 and lactate. The gases CO_2 and CH_4 are lost through eructation, lactate is generally used at rumen level by rumen microorganism, while VFA are absorbed through the rumen wall and transferred to the liver and then to the general circulation. Acetic, propionic and butyric acid are the most important VFA. They are produced by the fermentation of carbohydrates not only in the forestomach of ruminants (Van Soest, 1994) but, also, in the large intestine of the ruminant and non-ruminant mammalians (Montagne et al., 2003; Aluwong et al., 2010).

The amount and proportion of VFA produced during the fermentation process is strongly affected by the carbohydrate sources present in the diet. Of particular importance is the production of acetate and propionate. Fiber favours acetate production, whereas simple sugars and starch favour the production of propionate (Linington et al, 1998).

Acetate or acetic acid ($C_2H_4O_2$) is usually the major end-product of the carbohydrates fermentation (Annison and Linzell, 1964), it is produced in all mammals (Aluwong et al., 2010), where it has a role on the maintenance of the homeostasis (Shimazu et al., 2010). It can be considered as the most important precursor for lipogenesis in ruminant species, especially of milk fat synthesis (Church, 1988; Van Soest, 1994; Aluwong et al., 2010). Insufficient fiber levels in the diet are related with rumen acidosis and low

milk fat concentration in the milk (Van Soest, 1994). Acetate is also an important metabolic fuel. There are indications that acetate requirements of dairy sheep are particularly high, because they produce milk with very high fat content (Cannas et al., 2002).

Butyric acid ($C_4H_8O_2$) is very important as metabolic fuel to produce ATP (Linington et al., 1998) and it is also converted to ketone bodies, and in particular to β-hydroxybutyric acid (**BHBA**), used for fatty acid synthesis in the liver and mammary gland (Church, 1988).

Propionic acid ($C_3H_6O_2$) can be considered as the most important precursor for gluconeogenesis. Indeed, propionic acid is quantitatively transformed to glucose in the liver in the gluconeogenesis process, and it is by far the main source of glucose for ruminants (Ørskov, 1986; De Koster and Opsomer, 2013). Many studies suggested that glucose is the most important nutrient used by the mammary gland for lactose and milk synthesis (Elliot, 1980; Bell and Bauman, 1997; Aguggini et al., 1998; Pulina et al., 2005; Aluwong et al., 2010; De Koster and Opsomer, 2013).

As reported in the literature, the uptake of dietary glucose at intestinal level is limited in ruminant species. For this motivation, in ruminant VFA and especially propionate, are more effective than glucose in the stimulation of insulin production (Baumgard et al., 2016).

1.2 Carbohydrates, rumen function and milk production in small ruminants

Carbohydrates play a major role in the nutrition of ruminants, which, being herbivores, use large amounts of forages and by products rich in fiber and concentrates rich in starch.

Maintaining an optimal starch and NFC to NDF ratio in the diets is important for high milk yield in dairy cattle (Allen, 1997) because starch, or more in general NFC, are the most important energy substrates to sustain gluconeogenis in the liver and microbial activity at rumen level. In fact, the greater the amount of starch degraded in the rumen, the greater the amount of microbial N that flows in the duodenum (Firkins et al., 2001; Dann et al., 2014). Fiber is necessary for proper rumen function and to avoid reduction

of dietary degradation and microbial synthesis due to low pH. It is well known that a diet too rich in NDF would be not completely eaten, for its filling effect at rumen level, and a diet too poor in fiber, especially when combined with high-starch or NFC concentration, would cause a decrease in rumen pH and thus, likely, sub-acidosis or acidosis.

Another important aspect regarding carbohydrate utilization in small ruminants is if there are differences between dairy ewes and dairy goats in the optimal dietary starch and NDF content if the lactation stage affects the source of carbohydrate to be used.

As pointed out by Peel and Bauman (1987), during the lactation a homeorhetic regulation occurs. While in early lactation ruminants are stimulated to use glucose for milk synthesis, due to the dominance of the growth hormone control in energy partitioning, later on insulin becomes more important, favoring the utilization of metabolic fuels in the direction of body reserves accumulation than towards milk production. Based on these assumptions, the utilization of starch rich diets in the first half of the lactation should favor propionate production at rumen level and then gluconeogenesis in the liver, with positive effects on milk production. In the second half of the lactation starch rich diets should favor body reserve accumulation, for the increase in hematic glucose and thus the stimulation of the insulin action, while diets richer in digestible fiber should be able to provide energy without stimulating too much insulin action. Indeed, the fermentation of fiber produces large amount of acetate, which is a metabolic fuel not controlled by insulin and that can spare glucose. While this phenomenon might not be so important in dairy cows, due the fact that the intense genetic selection to which they were subjected increased the persistency of growth hormone action in favour of milk synthesis (Peel and Bauman, 1987), dairy sheep and dairy goats might be more affected by high-starch concentration in mid-late lactation, since they have been subjected to a much less intense genetic selection, especially dairy sheep, and thus are likely more sensitive to the insulin effects in favour of glucose utilization for body reserve accumulation (Cannas et al., 2002). Thus, it would be important to better understand the implications of the homeorhetic control of lactation in dairy small ruminants and to test if NFC, and specifically starch, can impact their

milk production in mid-late lactation, when likely they are more prone to insulin action, and if the metabolic response differ between ewes and goats.

1.3 Utilization of carbohydrates in mid-late lactation: comparison between goats and sheep

As said before, no reference values are available for dairy goats and ewes in terms of dietary concentration of starch, and more in general of NFC and of fiber. A particularly interesting aspect is the interaction between the type of carbohydrates and the homeorhetic control of lactation, especially in mid-late lactation, when the small ruminants enter a stage in which the partitioning of dietary energy towards milk production or energy reserves accumulation is controlled by insulin and not more by growth hormone.

On this regard, several authors observed the positive effect of high-starch diets in early lactation on the reduction of negative energy balance and on the increase of milk production in dairy ewes (Cannas et al., 2002; Bovera et al., 2004), goats (Hart, 1983) and dairy cows (Chagas et al., 2009). The positive effect of high-starch diet seems to be linked to the production of propionic acid. Propionic acid derived from starch fermentation is an important glucose precursor that is involved in the hepatic gluconeogenesis and increases the amount of glucose that can uptaken by the mammary gland, this favoring milk production.

In opposition, Cannas et al. (2002) suggested that in dairy sheep the utilization of high-starch diets in mid and late lactation might favor the partitioning of dietary energy in favor of body reserves accumulation, penalizing instead milk production. The same authors suggested that the substitution of dietary starchy feeds with feeds rich in fiber highly digestible and with limited filling effects, such as beet pulps, soy hulls and citrus pulps, would favor, in the same lactation stages, milk production rather body reserves accumulation. Cannas et al. (2002) also suggested that the same positive effects of fiber could not be observed when long forages with medium-high NDF values were used, since they would reduce the intake due to their high filling effects, and then cause marked decreases in milk production. They based these statements on the literature available at the time of their publication. The same research group could not confirm the

favorable effects of the substitution of starchy feeds with source of digestible fiber in goats, as reported by Cannas et al. (2007), suggesting that this species is more prone to use starch for milk production than dairy sheep.

To evaluate these assertions, studies in which low and high-starch diets were compared in mid and late lactation were reviewed. Those studies summarized the most important information on dietary composition and productive responses for dairy goats (Table 1) and dairy sheep (Table 2). All the studies selected compared high-starch diets rich in grains with low-starch diets rich in feeds with high fiber digestibility and small particle size.

The studies reviewed on goats (Table 1) showed that most of the times the substitution of starchy grains with feeds rich in digestible fiber resulted in equal or even higher DMI, probably as a result of the effort of the goats to compensate the lower energy concentration, in comparison to starchy grains, of digestible fiber-rich feeds. The higher intake could be achieved due to the small particle size and the limited filling effects of these feeds. Milk production was higher for the high-starch diets in 3 studies and did not differ in the other studies. Milk fat concentration was almost always numerically or statistically highest with the low-starch, high digestible fiber diets, while milk protein and lactose concentrations were little affected. The variation in BW were numerically or statistically highest in the high-starch diets.

The studies reviewed on dairy sheep (Table 2) showed that, similarly to goats and for the same reasons, most of the times the substitution of starchy grains with feeds rich in digestible fiber resulted in equal or even higher DMI. Milk production was significantly higher in all but one study (in which the differences were not significant) in the low-starch, high digestible fiber diets. Similarly to goats, milk fat concentration was almost always numerically or statistically highest with the low-starch, high digestible fiber diets, while not clear patterns could be observed for milk protein and lactose concentrations. The variation in BW and BCS were significantly higher, in favor of high-starch diets, only in one study.

Thus, it appears that dairy goats and dairy sheep have clearly distinct patterns in the utilization of starch and digestible fiber during mid and late lactation, with milk production favored by high-starch diets in goats and high digestible fiber diets in ewes.

The mechanisms behind this impressive difference could be related to a different feeding behavior and thus rumen utilization of the carbohydrates between goats and sheep, to different metabolic requirements for glucose and, possibly, acetate, or to different, in entity or time, hormonal regulation of energy partitioning during lactation. These possible mechanisms will be explored in the subsequent paragraphs.

1.4 Nutrient partitioning during the lactation

Nutrient partitioning can be defined as the pattern of energy deposition, realized by the liver to satisfy the nutrients requirements of animals (Bauman and Currie, 1980). Generally, in animals with high requirements, energy utilization is oriented toward fetus or mammary gland, in pregnant and lactating animals respectively (Bauman and Currie, 1980), whereas when the requirements of animals are lower, energy flues towards peripheral tissues, in adipose tissue in particular.

Homeostasis and homeorhesis mechanisms are involved in the partitioning of nutrients in the body tissues (Bell and Bauman, 1997), the first through maintenance of an equilibrium and the second through maintenance of a change (Bauman and Currie, 1980).
According to Bauman and Currie (1980) the homeostasis is defined as "*maintenance of physiological equilibrium, i.e. constant conditions in the internal environment*" and thus the tendency to maintain a stable internal environment and constant during time. Homeorhesis is instead defined as "*Orchestrated changes for the priorities of a physiological state, i.e. coordination of metabolism in various tissues to support a physiological state*".
In lactating animals, homeostatis and homeorhesis control energy partitioning to drive nutrient fuel toward mammary gland or to body reserves. This control occurs in response to the energy balance (energy supply:energy requirements), that can be

negative or positive. So, during early lactation energy is preferentially directed to mammary gland whereas from mid-lactation, animals need to recover body reserves mobilized in early lactation and energy is used for this purpose (Bauman and Currie, 1980).

The most important nutrient that are directed to mammary gland or body reserves is glucose, a key element essential for milk production because is the most important precursor, in the epithelial cells, for lactose synthesis and it is involved, also, in the ATP production and in the protein, lipid and nucleotide synthesis (Zhao and Keating, 2007). In addition, it is a nutrient that is absorbed from adipocyte when the energy of the diet exceeds the requirements of the animals, in the common phenomena called lipogenesis. Both cases, gluconeogenesis and lipogenesis, are associate to high carbohydrates diets.

1.4.1 Factors influencing nutrient partitioning

Nutrient partitioning toward mammary gland or body reserves is regulated by hormones (Sasaki, 2002). Hormonal changes or changes in the number of receptors are responsible of this partitioning (Collier et al., 1984). Generally, changes of the hormonal status during lactation occurs in a long-term than the changes that occurs during the transition period from pregnancy to lactation (Collier et al., 1984).

Hormones involved on milk production can be grouped into three classes: reproductive hormones (estrogen, progesterone, placental lactogen, prolactin and oxytocin), metabolic hormones (growth hormone or somatotropic hormone, corticosteroids, thyroid hormone and insulin) and mammary hormones (growth hormone, prolactin, parathyroid hormone-related peptide (PTHrp) and leptin) (Neville et al., 2002).

Most important hormones involved in the energy partitioning between milk production and body reserves are metabolic hormones such as growth hormone (Peel and Bauman,1987) and insulin (Tucker, 2000) that can change milk synthesis altering the partitioning of nutrients to the udder (Neville et al., 2002). Some authors suggested that they follow an opposite course during lactation and when the blood concentration of growth hormone is high, the nutrients are directed to udder, contrariwise when its blood concentration is low (Peel and Bauman,1987). These changes are associated with insulin resistance status during early lactation and insulin sensitivity status in late

lactation. Then, nutrient partitioning is an equilibrium of metabolic status where nutrients are directed toward mammary gland or peripheral tissues.

So, to drive nutrient partitioning towards mammary gland is important that other factors inhibit nutrient uptake by the adipose tissue. In particular, the role of adipose tissue and mammary gland metabolism as well as the hormonal and metabolic mechanisms that regulate energy partitioning will be addressed in detail. Hence, an overview of the most important hormones associated with nutrient partitioning towards body reserves or milk production is provided below.

- ***Insulin***

Insulin is a peptide hormone, with anabolic properties, produced by the β cells of the pancreas, that play an important role on metabolic regulation (Sasaki, 2002) and in carbohydrate, lipid and protein metabolism (Baumgard et al., 2016).

Insulin secretion depends on many factors, with blood glucose concentration being the most important (Aguggini et al., 1998). In ruminants, where glycemic values are low, the most important factors that stimulate insulin activity are glucose precursors, such as propionic acid, glycerol, lactate and amino acids (Aguggini et al., 1998; De Koster and Opsomer, 2013). In particular, insulin is one of major regulators of glucose metabolism because it is able to act on different body tissues such as liver, mammary gland, adipose tissue and muscles (Sasaki, 2002) and balance the availability and the demand of nutrients (Samuel and Shulman, 2012). It has a hypoglycemic effect, assures an optimal glycemia removing glucose from blood circulation when its level exceeds the optimal range (Aguggini et al., 1998; Duque-Guimarães and Ozanne, 2013), increasing cellular glucose uptake (Baumgard et al., 2016) by muscle and adipose tissue (Brockman, 1978), cells differentiation and growth, stocks of fats through lipogenesis, protein and glycogen reserves (Brockman, 1978), and inhibit gluconeogenesis by the liver, lipolysis, glycogenolysis, both in ruminants that in non-ruminants (Hart, 1983; Saltiel and Kahn, 2001; Drackley et al., 2001; Baumgard et al., 2016). Among these roles, the most important is to allow nutrient deposition (Rosi et al., 2009) but also the storage of metabolites in the peripheral tissues (Brockman, 1978); it can be considered as the most important factor responsible of fatness and lipogenesis (Aguggini et al., 1998; Kersten,

2001). It drives nutrient fluxes to peripheral tissues such as muscles or adipose tissue (Brockman, 1978; Debras et al., 1989). Some studies suggested that the response to insulin is lower in ruminant compare with non-ruminant species (Brockman, 1978) and its action is antagonized by growth hormone, glucagon, adrenalin and glucocorticoids (Veerkamp et al., 2003).

Insulin and milk production

Several studies observed an inverse relationship between insulin and milk production (Lomax et al., 1979). As reported by Debras et al. (1989), even though insulin is required to maintain lactation, it does not stimulate glucose uptake by the mammary gland (Debras et al., 1989). In particular, at the end of pregnancy and during lactation, blood insulin levels and the response of body reserves to insulin are low (Lomax et al., 1979; Karapehlivan et al., 2007). The reduction of insulin levels in early lactation is a metabolic adaptation to reduce glucose availability for body tissue and to satisfy nutrient requirements of the udder during lactation (Bizelis et al., 2000; Aschenbach et al., 2010), increasing the glucose uptake from the mammary gland, which is not insulin-responsive (van Knegsel et al., 2007). In lactating animals, as reported by Adewuyi et al. (2005), the reduction of blood insulin is an indicator of negative energy balance, during which insulin sensitivity of tissues decreases (Chilliard, 1992; Karapehlivan et al., 2007). This is due to the phase of insulin resistance that occurs in early lactation, during which glucose absorption in muscle and adipose tissue is inhibited, while the transfer to mammary gland or toward non-insulin dependent tissues is favored (Chilliard, 1992; Regnault et al., 2004; De Koster and Opsomer, 2013; Fiore et al., 2014). In other words, if fat mobilization occurs when insulin is not acting, the increase of free fatty acids can even inhibit insulin production by the pancreas (Regnault et al., 2004; Karapehlivan et al., 2007; Fiore et al., 2014). In particular, in early lactation insulin seems to be involved in the reduction of hepatic gluconeogenesis or in the decrease of glucose production and can be linked to a mechanism to spare glucogenic substrates (Debras et al., 1989).

Insulin levels begin to rise with the progress of lactation, when milk production decreases (Lomax et al., 1979; Vicini et al., 1991; Bell and Bauman, 1997; Sasaki,

2002; Fiore et al., 2014). For example, in Tsigai ewes monitored at 20, 40 and 60 days of lactation, glucose and insulin levels increased over time (Antunović et al., 2011). The increase of blood insulin in mid-lactation compared to early lactation was also observed in another study, where an euglycemic-hyperinsulinemic test was conducted in Alpine goats (6-7 years old) in early lactation (15-26 DIM), mid-lactation (78-91 DIM) and during the dry period (169-194 days postpartum) to evaluate the role of insulin in the partitioning of nutrients between mammary gland and other sites (Debras et al., 1989). The experiment showed that basal plasma glucose did not change during lactation, but it was higher during the lactation than the dry period; basal plasma insulin did not differ in early lactation and in the dry period but increased in mid-lactation. In addition, insulin infusion had a greater effect, on the reduction of blood glucose, in early than mid-lactation (Debras et al., 1989). In dairy cows, insulin concentration remained low until the 7 month of lactation (Accorsi et al., 2005) and increased with the advancement of lactation and it was negatively related with the growth hormone concentration, which instead decreased (Koprowski and Tucker, 1973; Accorsi et al., 2005). Herbein et al. (1985) observed that in dairy cows GH:insulin ratio decreased with the advancement of lactation.

Insulin and genetic selection

Blood insulin concentration seems to be associated to the productive level of animals; in fact, Hart et al. (1978) demonstrated that insulin concentration was higher in lowly productive dairy cows than in highly productive dairy cows, according to what reported by Gutierrez et al. (1999). In low productive animals, insulin is high and the high insulin sensitivity drives glucose to adipose tissue. In other cases, however, it did not differ between lactating high and low genetic animals (Barnes et al., 1985; Westwood et al., 2000; Veerkamp et al., 2003).

Insulin and diet

Metabolic changes of insulin and other hormones could be linked to the carbohydrates of the diet. It is well known that insulin action is stronger with high NFC diets during the lactation of ewes (Metcalf and Weekes, 1990; Cannas et al., 2013) and in goats

(Wang et al., 2016). In fact, propionate derived from NFC fermentation seems to stimulate insulin production, in opposition to what observed for acetate (Brockman, 1978).

In an experiment conducted in Saanen goats in mid-lactation, fed high (33.2 %) and low-starch diets (17%), plasma insulin and insulin-like growth factor I (**IGF-I**) were higher in the high-starch than in the low-starch group (Rosi et al., 2009). The concentration of the same hormones was highest also in the milk of goats fed a high-starch diet (Rosi et al., 2009). It seems that high-starch diets can directly increase ruminal propionate (Rosi et al., 2009), plasma insulin and glucose (Voelker and Allen, 2003; Rosi et al., 2009) and indirectly IGF-I, which was increased consequently to insulin concentration (Rosi et al., 2009). In another experiment conducted in mid lactating Saanen goats, the supply of high-starch diets increased IGF-I (9.53 vs. 6.88 nM) and insulin concentration (93.5 vs. 75.6 pM) compared to low-starch diets ($P<0.05$) (Magistrelli et al., 2005). Similarly, blood insulin concentration was higher in ewes in mid-lactation fed a low NDF-high starch diet than in ewes fed a high NDF-low starch diets (27.0 vs. 17.6 μU/ ML, respectively for low and high NDF diets; $P<0.001$) (Cannas et al., 2004). In contrast in another experiment in mid lactating ewes, fed a high and low-starch diets, blood insulin concentration was higher in ewes fed a low-starch diet than in those fed a high-starch diet (18.24 vs. 13.43 U/ml; $P=0.009$) (Cannas et al., 2013). As commented by the authors, this result was difficult to explain.

It brief, insulin can be considered as the most important hormone involved in the energy partitioning: it is stimulated by high-starch diets, increases during lactation and favors fat deposition or body reserves accumulation, in contrast to milk production. In addition, in high productive animals, insulin is low and associated to an insulin resistance status that favors glucose uptake by mammary gland.

- ***Growth hormone***

Growth hormone (**GH**) or somatotrotopic hormone (**STH**) is a proteic hormone, discovered in 1920 by Evans and Simpson, that is synthesized and secreted from the anterior pituitary gland (Etherton and Bauman, 1998). Many biological effects of GH are mediated by a group of peptides, somatomedins or insulin-like growth factors

(**IGF**), produced in the liver through GH stimulation (Aguggini et al., 1998; Petitclerc et al., 2000). GH activity depends, also, by two peptides: growth hormone-releasing factor (GFR), in a positive sense, and somatostatin, which instead inhibits STH action (Etherton and Bauman, 1998). It can be considered as an ubiquitary hormone because it is able to influence glucidic, lipidic and proteic metabolism. In opposition to insulin, GH has a catabolic metabolism oriented toward reserve mobilization (Aguggini et al., 1998).

Regarding glucose metabolism, it inhibits cell glucose uptake with a hyperglycemic effect (Aguggini et al., 1998). In lipid metabolism, it favors lipid mobilizations and inhibits lipogenesis (Aguggini et al., 1998). Several studies suggested that GH is important during growth, pregnancy and lactation. In fact, it influences positively body growth and in particular cell proliferation (Etherton and Bauman, 1998), drives nutrient partitioning toward fetus during pregnancy and improves milk production during lactation (Aguggini et al., 1998).

Growth hormone and milk production

In ruminants, GH is very important for the galactopoietic activity because improves milk production (Neville et al., 2002) and it is considered the key hormone for milk production (Chilliard, 1992; Svennersten-Sjaunja and and Olsson, 2005).

In dairy cows, the mammary gland can synthesize GH receptor and has been defined as GH target tissue, due to the expression of GH receptor in the alveolar epithelial cell (Glimm et al., 1990; Hovey et al., 1999).

In United States, several studies observed that in bovine selected for milk production, the use of GH injected to the animals increase milk production more than 20%. In addition, the same treatment increases hepatic gluconeogenesis (Bell and Bauman, 1997).

The mechanism behind the activity of GH on milk production is not well defined. From a metabolic point of view, probably GH increases the availability of milk precursors stimulating lipolysis and glucose partitioning from body reserves to the mammary gland (Hart, 1983; Davis and Collier, 1985). In fact, several studies suggested that GH favors milk yield through body reserves mobilization and glucose partitioning towards

mammary gland, increasing milk precursors availability for the udder (Welt and Wilhelmi, 1950; Luft and Guillemin, 1974; Hart, 1983).

In addition, in analogy to what observed for insulinic hormone, its concentration changes during the lactation: GH concentration is high in early lactation and decreases during lactation in opposition to the trends observed for insulin concentration, which instead increases during lactation (Petitclerc et al., 2000). In fact, high GH levels inhibit the lipogenic action of insulin (Bauman and Vernon, 1993; Bell and Bauman, 1997). Then, in early lactation, with high GH concentration, adipose tissue is insulin resistant (Bell and Bauman, 1997; Petitclerc et al., 2000). With the advancement of the lactation, GH concentration decreases, milk production is reduced, in association to a status of insulin sensibility. In dairy cows, GH concentration remain high until the 3 month of lactation (Accorsi et al., 2005). However, in the literature at the best of my knowledge there are not reports describing the evolution of GH during the whole lactation of dairy ewes and goats. The information available is fragmented to specific stages and short periods, as reported by Cannas et al. (2002). Thus, it is difficult to interpret the interaction between carbohydrate type and stage of the lactation.

Growth hormone and genetic selection

The production level can have an effect on GH. As suggested by several studies, GH plays a crucial role in the partitioning of nutrients toward milk production but only if milk production is sufficiently high, while the effects are limited if the animals are not much productive (Hart et al., 1978; Hart, 1983). As suggested by Kazmer et al. (1986), high GH concentration is the results of the intense genetic selection. In fact, in highly yielding cows involved in the intense genetic selection, the content of blood GH generally is high as confirmed by several authors (Hart et al., 1978; Barnes et al., 1985; Kazmer et al., 1986; Veerkamp et al., 2003; Weber et al., 2007); however, others studies did not find differences (Diab and Hillers, 1996; Veerkamp et al., 2003). Certainly, small ruminants, and especially dairy sheep, have been less selected than dairy cows for milk production. Thus, more ancestral homeorhetic controls are expected, possibly making this species more sensitive to insulin action.

Growth hormone and diet

Blood GH concentration is certainly affected by dietary carbohydrates in mid-late lactation. GH concentration in mid lactating goats was higher for high amylose starch (with low rumen degradability) than for normal starch diets, at equal dietary starch concentrations (2.55 vs. 1.65 ng/ml; P=0.06), suggesting that high-starch degradability negatively impacts GH concentration (Wang et al., 2016). The increase of blood GH (P<0.005) during the lactation was associated to the decrease of the NDF of the diet in browsing goats monitored monthly from January to June (Juárez-Reyes et al., 2008). However, this could have been the result of the increased day length, which it has been shown to increase GH secretion (Jin et al., 2012). In mid lactating ewes fed diets containing different forage:concentrate ratios (90:10, 75:25, 60:40), blood GH concentration was highest in the ration having the 90:10 forage:concentrate ratio (Bomboi et al., 2002). In another experiment, mid lactating ewes fed a high NDF diets had higher blood GH concentration than the ewes fed a low NDF diet (1.43 vs. 1.23 ng/ml; P< 0.021) (Cannas et al., 2004). However, this pattern is not always evident, since in another study high digestible fiber diets did not differ in blood GH concentration compared to high-starch diets (Cannas et al., 2013).

Thus, in summary it seems that GH concentration has an important role in nutrient partitioning, driving nutrient flux to the mammary gland and mobilizing body reserves. In addition, it changes in relation to lactation stage, being GH higher in early than late lactation, and in relation with the production level, being higher in highly than lowly productive animals. In addition, it seems that in mid-lactation it changes in relation with the diet, being higher with high-starch or more fermentable diets in both in goats and ewes.

- ***Insulin-like growth factor I***

IGF-I is a hormone included in the IGF system, a group of peptides. Some of them are considered GH mediators and are represented by: IGF-I, IGF-II, IGF receptors I and II, IGF-binding protein (IGFBPs) and IGFBO proteases (Veerkamp et al., 2003). In particular, IGF-I is 100% GH dependent (Zulu et al., 2002). In fact, the somatotropic hormone stimulates the liver to produce IGF-I, even though it can be produced locally,

such as in the mammary gland (Cohick, 1998). Indeed, some IGF-I receptor were identified in the ovine mammary gland (Akers, 2002).

Insulin-like growth factor I and milk production

In ruminant species, IGF-I is linked to the galactopoietic activity and can stimulate milk production even in the absence of increased GH secretion (Dahl et al., 1997). In fact, it seems that the increase in milk production of cows exposed to long photoperiod is controlled by IGF-I (Dahl et al., 1997). In addition, when infused into the mammary gland it increased milk secretion in the rate of 25 ± 6% in dairy goats (Prosser et al., 1990). However, as suggested by Cohick (1998), little evidence support that IGF-I is involved in the galactopoietic activity. Taylor et al. 2004, observed in dairy cows that milk yield was negatively associated with plasma IGF-I concentration ($P<0.05$) and was higher in the primiparous than multiparous animals ($P<0.001$), before and after calving, due to differences in the age, being IGF-I a hormone that changes with the age.

In analogy to what observed for other hormones, IGF-I changes during the lactation and in particular in relation with the energy balance. It is low in early lactation (negative energy balance) (Sharma et al., 1994; Taylor et al., 2004) and increases to peak at the end of lactation (positive energy balance) (Abribat et al., 1990; Vicini et al., 1991; Sharma et al., 1994; Dahl et al., 1997; Taylor et al., 2004). In contrast, IGF-2 is not influenced by the lactation stage (Vicini et al., 1991; Cohick, 1998).

In early lactation, IGF-I concentration is low because probably the negative energy balance controls the synthesis of IGF-I from the liver. Thus, IGF-I and can be used as nutritional indicator in early lactation (Zulu et al., 2002; Taylor et al., 2004). Considering that it is a hormone that favors lipogenesis and not lipolysis (Aguggini et al., 1998), and that animals with positive energy balance have a tendency to deposit fat reserves, it is logical to think that IGF-I tends to have high concentrations at the end of lactation. This evolution is in accordance to what reported by Oliveira et al. (2011), who suggested that even though IGF-I is secreted trough GH action, GH decreases insulin sensibility whereas IGF-I increases it. In addition, it seems to be positively associated with metabolites and hormones that typically increase in positive energy balance, such

as glucose and insulin or with body weight and BCS, whereas it is negatively associated with NEFA concentration (Zulu et al., 2002).

Insulin-like growth factor I and genetic selection

Some studies suggested that IGF-I is low in high genetic merit cow (Veerkamp et al., 2003; Weber et al., 2007) even though these information are not enough. In high genetic merit cows, plasma IGF-I concentration was lower than in medium genetic merit cows (130.4 vs. 144.0 ng/ml; P=0.07) (Snijders et al., 2001).

Insulin-like growth factor I and diet

Several studies suggested that nutritional status plays an important role in the regulation of IGF1 hormone (Zulu et al., 2002). Being the negative energy balance (**NEB**) a state of under nutrition, IGF-I values are lower in early lactation and start to increase with the advancement of the lactation, when the energy balance becomes positive (Zulu et al., 2002). For the same motivation, IGF-I can be considered a nutritional indicator (Zulu et al., 2002). IGF-I concentration decreased in dairy sheep subjected to short-term feed restriction (50% of nutrient requirements) (Pulina et al., 2012) and it was lower in ewes with low BCS (BCS < 2.5; BW 36.2 ± 4.7 kg) than with high BCS (BCS > 2.5; BW 48.8 ± 5.4 kg). In another experiment, the supplementation of concentrate tree times per day and the use of high quality forage increased IGF1 concentration in late lactating ewes (Chestnutt and Wylie, 1995).

Not only the nutritional status but also the type of the diet and in particular the type of carbohydrate affects the evolution of IGF-I differently in sheep than in goats. In fact, Magistrelli et al. (2005) observed a higher IGF-I concentration in mid lactating Saanen goats fed a high-starch diet (33% starch, on a DM basis; IGF1: 93.50 pM) compared to low-starch diet (17% starch, on DM; IGF1: 75.60 pM), whereas Cannas et al. (2004) observed the highest IGF-I concentration in Sarda lactating ewes fed a highly digestible fiber (71.8 vs. 66.4 ng/ml; high and low NDF diets; respectively). Overall, the literature available does not allow to clarify if there are differences between ewes and goats or due to their diet.

- ***Leptin***

Leptin is a proteic hormone synthesized primarily in the white adipose tissue (Zhang et al., 1994; Ahima and Flier, 2000; Ingvartsen and Boisclair, 2001; Accorsi et al., 2005) and in the placenta, intestinal tract, mammary gland, fetal tissues, muscle etc. (Houseknecht et al., 1998; Prolo et al., 1998; Chilliard et al., 2001). It was discovered in 1994 as secretion product of the *ob* gene in rodents (Prolo et al., 1998; Chilliard et al., 2001). The expression of leptin on adipose tissues in ruminant species was studied since 1997 (Chilliard et al., 2001).

It is secreted in proportion to the presence of adipose tissue (Prolo et al., 1998), and if it is present in high amounts, it reduces the feed intake through the inhibition of the Y Neuropeptide (NPY, responsible to increase of appetite) in the hypothalamic region (Zhang et al., 1994; Vernon and Sasaki, 2001). In other words, it is able to send a signal to the brain (Prolo et al., 1998) when the amount of adipose tissue increases, decreasing its growth (Miner, 2004; Rosi et al., 2009). Then, leptin can regulate the sense of satiety, in contrast with ghrelin, which instead stimulates the appetite. Feed intake decreases in obese sheep, probably due to the compounds released from adipose tissue that inhibited feed intake or to the increase in omental and mesenteric fats that reduced the space occupied by the rumen (McCann et al., 1992).

As reported by Houseknecht et al. (1998), the discovery of leptin and the demonstration that leptin could reduce obesity in mouse, increased the attention of scientific community towards this hormone. As reported by several authors, plasma leptin is an indicator of the body fatness in ruminants and variations in plasma leptin are linked more to the variations in body fat mass than to changes of the nutritional status (Delavaud et al., 2000).

In sheep, plasma leptin was correlated with body fat ($r=+0.68$; $P<0.001$) and BCS ($r=+0.72$; $P<0.001$) (Delavaud et al., 2000). In Criollo goats, BCS affected plasma leptin, which increased simultaneously with the increase of BCS (Gámez-Vázquez et al., 2008). In beef cattle, leptin was a good indicator of the body composition and in particular a predictor of adiposity (Geary et al., 2003), whereas in sheep it was associated to adipose tissue mass and its production was sexually dimorphic (Blache et al., 2002). Other studies suggested that plasma leptin in associated to the subcutaneous

fat (Montague et al., 1998; Maury and Brichard, 2010) in sheep (Kumar et al., 1998) and goats and with visceral fat in cows (Chilliard et al., 2001),

It is well known that leptin favors fatty acid oxidation (Minokoshi et al., 2002; Maury and Brichard, 2010), glucose uptake (Maury and Brichard, 2010), and inhibits lipid storage (Maury and Brichard, 2010); as reported by other authors, leptin is involved, also, in the regulation of body temperature (Whitley et al., 2005), in the reproduction and in the regulation of body weight (Prolo et al., 1998). One of the most important factors that can regulate leptin level is the hormone insulin (Houseknecht et al., 1998). Adewuyi et al. (2005) observed that blood leptin was positively correlated with insulin and glucose concentrations, whereas it was negatively correlated with growth hormone and NEFA.

The secretion of leptin can be pulsatile but increasing the number of samplings does not improve the accuracy of its quantification, due to uncontrolled random effects of its pulsatility (Blache et al., 2002).

Leptin and milk production

In ruminants, leptin is positively associated with their energy balance; leptin levels are low at the beginning of lactation (Accorsi et al., 2005), when the energy balance is negative, due to energy deficit and in particular to fat mobilization (Reist et al., 2003; Accorsi et al., 2005) or too low insulin level and too high GH concentration (Block et al., 2001). Low blood leptin concentration in early lactation is the result of an adaptation that permits to reduce the tissues's insulin sensitivity and to drive blood glucose to the mammary gland (Bell and Bauman, 1997; Etherton and Bauman, 1998). In analogy to what observed for insulin and IGF-I, leptin level start to increase with the advancement of lactation. In dairy cows, leptin concentration starts to increase from the fourth to the six month of lactation (Accorsi et al., 2005).

Milk leptin was found in the goat milk and was negatively correlated with the days postpartum, in particular when days postpartum increased, milk leptin decreased ($P<0.001$), whereas blood leptin was not associated with days postpartum. In addition, milk serum leptin and blood serum leptin were not correlated (Whitley et al., 2005). Leptin is expressed in the mammary gland of cows, goats and sheep (Chilliard et al.,

2001). In sheep, in particular, its expression changes during lactation (Laud et al., 1999; Chilliard et al., 2001).

Leptin and genetic selection

In the selection program, leptin concentration is associated to the carcass genetic merit, as observed in Angus steers (Nkrumah et al., 2007). In particular, the expression of the *ob* gene controls the quality of the carcass (Lasagna et al., 2008).

Leptin and diet

The relationship between leptin and the carbohydrates of the diet is unknown or not well defined. No differences were observed in plasma leptin levels in mid-lactating dairy sheep fed a high or low highly digestible fiber (Cannas et al., 2004) or in dairy goats fed a normal or high amylose starch, at equal dietary starch concentrations (Wang et al., 2016). The nutritional status seems to be the major factor that influence the concentration of leptin; in particular, under-nutrition seems to reduce blood leptin levels and this response occurs as a strategy to stimulate the re-feeding behavior (Chilliard et al., 2001). However, in late-lactating goats replacing a part of concentrate of the diet with soybeans did not change leptin mRNA expression in the subcutaneous or perirenal fat, even though energy balance decreased (Chilliard et al., 2001). Other studies observed that blood leptin is linked more with the body fat mass than the nutritional level (Delavaud et al., 2000), even though in the long term high body fat mass depends by positive nutritional status.

1.5 Practical implications

The review of the literature poses two important questions:

a) Can different carbohydrate sources modulate differently the hormonal status of sheep and goats?
b) Do sheep and goats differ for their hormonal status during the lactation, independently by the diet used, and is this linked to their production level or milk composition?

Ewes

As for all dairy ruminants, during mid-lactation sheep reduce their milk production and replenish the body reserves mobilized in early lactation. If high-starch diets are used, the extra energy supplied to this species in mid-lactation seems to be used only to accumulate body fat, probably due to their very low GH concentration during all lactation, making them very sensitive to insulin (Cannas et al., 2002). In fact, sheep compared with other ruminants have been subjected to less intense genetic selection compared to dairy cows and possibly dairy goats, where GH is higher and more persistent compares to sheep (Peel and Bauman, 1987; Sorensen et al., 1998). The higher persistency of milk production in sheep fed highly digestible fiber sources can be explained by the fact that acetate, produced in large amounts during fiber fermentation, is not only a precursor of fat but also an important energy fuel that is not controlled by insulin. Thus, highly digestible fiber diet may help to spare glucose, especially at mammary gland level, without stimulating the action of insulin, to which sheep in this stage are very sensitive (Cannas et al., 2002). Lactating ewes probably have a higher acetate requirement than lactating cows, because their milk has much higher fat to lactose ratio. Pethick and Lindsay (1982) found that acetate uptake by the lactating ewe udder represents a greater drain on acetate supply than the udder of dairy cows. This suggests that they might be able or prone to use acetate also as an energy source to spare glucose than can be used to produce lactose, necessary for milk synthesis (Cannas et al., 2002).

Goats

Compared with ewes, goats respond positively to high-starch diets in all stages of lactation, probably for their very high milk production even in mid-late lactation. The positive effect of high-starch diet can be due to need to produce glucose from propionate, to sustain milk lactose synthesis. Similarly to cows, goats produce a milk with a low fat to lactose ratio and are, therefore, probably less adapted to use diets that stimulate large acetate production. This species difference might be due to higher GH persistency in goats compare to sheep. However, this hypothesis has not been tested yet comparing the species in the same feeding conditions.

1.6 Conclusions and objectives of the research

In conclusion, the literature showed that the effect of different types of carbohydrates (starch or digestible fiber) is variable during the lactation between sheep and goats. In sheep, high-starch diet has a positive effect on milk production in early lactation but not in mid-lactation, while milk production is positively influenced by highly digestible fiber diet. In goats, high-starch diets seem to have a positive effect during the whole lactation.

These differences could be associated with variations in the GH:insulin ratio, that drives nutrient partitioning toward milk production or body reserves. Possibly high digestible fiber diet might avoid that the GH:insulin ratio decreases too much in late lactation and this might favor sheep more than goats, since the lower productivity of the former compared to the latter might result in different regulations of their hormonal status and different GH:insulin ratios.

If these mechanisms will be better understood, it will be possible to improve the performances of the animals modulating more accurately their dietary carbohydrate composition throughout the lactation and accounting for species and production level differences. Other implications relate to the beneficial effects of feed rich in digestible fiber on rumen environment, when used to substitute part of dietary starch. Since most feed rich in digestible fiber are by-products (e.g. soyhulls, beet pulps, citrus pulps, wheat brans), their utilization could also reduce feeding costs and the competition with nutrients than can be used also by humans, such as starch (Dann et al., 2014).

Thus, the main objectives of this thesis were to:

a) compare the effects of the prevalent types of carbohydrates of the diet, with focus on starch and digestible fiber, on the productive performances of dairy goats and sheep during mid and late lactation
b) explore possible nutritional, metabolic and hormonal differences, and the related underlying mechanisms, in carbohydrate utilization between dairy goats and dairy sheep

c) define the best combination of carbohydrates that favors dietary energy partition towards milk production, and thus enhances lactation persistency, during the intermediate stage of the lactation of dairy goats and dairy sheep

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3. TABLES

Table 1. Effect of high and low-starch diets, obtained substituting starchy grains with sources of digestible fiber, on milk performances and body reserves in goats during mid-lactation. Coding: ↑ significantly higher than the other treatment (P<0.05 or lower).

Breed	Diet	Stage	NDF (% DM)	NFC (% DM)	Starch (% DM)	DMI (kg/d)[a]	Milk (kg/d)	FCM (g/d)[b]	Fat (g/d)	Protein (g/d)	Lact. (g/d)[c]	Fat (%)	Protein (%)	Lactose (%)	BW var. (g/d)[d]	Ref.[e]
Saanen	Control diet (C)	mid-lactation	33.0	40.6		2.627	3.882		126	116.6		3.23	2.96	4.69		*1*
	Non forage diet (NF)		36.8	28.8		2.915	3.841		134	115.3		3.46 ↑	3.02	4.76		
	Control diet (C)	late-lactation	33.0	40.6		1.975	2.394		95.8	89.4		3.99	3.77	4.59		
	Non forage diet (NF)		36.8	28.8		2.028	1.889		94.4	73.3		5.00 ↑	3.91	4.14		
Saanen	HS diet	mid-late lactation	31.6	47.3	33.2	2.229	4.323					2.49	2.69	4.49 ↑		*2*
	LS diet		39.7	40.0	17.0	2.333↑	4.328					2.54	2.64	4.39		
Sarda	Low NDF diet	mid-late lactation	36.9	36.0		1.840	1.612 ↑		65.3 ↑	67.4 ↑		4.08	4.2		129	*3*
	High NDF diet		44.7	29.3		1.990 ↑	1.384		57.6	59.5		4.09	4.3 ↑		96	
Saanen and Alpine	High concentrate	mid-lactation	36.0		9.7	2.98	3.710 ↑	3680 ↑	128	116 ↑	173 ↑	3.49	3.20	4.67	59 ↑	*4*
	Low concentrate		40.9		4.4	2.68	2.950	3270	123	96	137	4.19 ↑	3.25	4.60	14	

Table 1. (continued).

Breed	Diet	Stage	NDF (% DM)	NFC (% DM)	Starch (% DM)	DMI (kg/d)[a]	Milk (kg/d)	FCM (g/d)[b]	Fat (g/d)	Protein (g/d)	Lact. (g/d)[c]	Fat (%)	Protein (%)	Lactose (%)	BW var. (g/d)[d]	Ref.[e]
Muciano-Granadina	Corn grain diet (CRN)	mid-lactation	34.8	37.00	28.0	2.04	2.240					5.44	4.04	4.74		*5*
	Soyhulls and gluten feed (SHGF)		47.5	20.0	6.6	2.10	2.110					7.02 ↑	4.07	4.71		
Muciano-Granadina	HS diet	mid-lactation	40.6	28.7	21.9	2.03	2.400 ↑					5.5	3.9	4.7		*6*
	LS diet		46.5	21.8	7.0	2.07	2.200					6.4 ↑	3.9	4.7		

[a] Dry matter intake; [b] fat-corrected milk yield; [c] lactose; [d] body weight variation; [e] references
[1] Bava et al., 2011; [2] Rapetti and Bava, 2004; [3] Cannas et al., 2007; [4] Serment et al., 2011; [5] López and Fernández, 2013; [6] Ibáñez et al., 2015

Table 2. Effect of high and low-starch diets, obtained substituting starchy grains with sources of digestible fiber, on milk performances and body reserves in ewes during mid-lactation. Coding: ↑ significantly higher than the other treatment (P<0.05 or lower).

Breed	Diet	Stage	NDF (% DM)	NFC (% DM)	Starch (% DM)	DMI (kg/d)[a]	Milk (kg/d)	FCM (g/d)[b]	Fat (g/d)	Protein (g/d)	Fat (%)	Protein (%)	Lactose (%)	BCS[c] var.	BW var. (g/d)[d]	Ref.[e]
Friesian	High NSC	from 122 to 199 DIM *			20.7 **	2.49	1.107	1.043			6.15	4.94			49.2 ↑	*1*
	Low NSC				15.7 **	2.61	1.209 ↑	1.186 ↑			6.39 ↑	4.86			28.7	
Sarda	High energy	mid-lactation	37.9	39.6	25.7	2.20	1.260		68.9		5.58		4.55			*2*
	Low energy		48.4	28.7	15.0	2.39 ↑	1.420 ↑		81.1 ↑		5.82 ↑		4.63			
Boutsiko	Concentrate with maize (C)		36.4	45.6		1.80	0.712	806	52.0	42.4	7.3	5.94	4.97		62	*3*
	Concentrate with soy hulls (SH)		49.4	32.7		1.80	0.733	936 ↑	61.0 ↑	44.9	8.3 ↑	6.15 ↑	4.99		61	
Sarda	High NSC	mid-lactation	38.6	39.9	26.1		0.900				6.71	5.02				*4*
	Low NSC		40.7	35.9	20.7		0.996 ↑				6.69	4.99				
Sarda	Low NDF	mid-lactation	24.5	45.9	33.4	2.506	1.646		120	92	7.3	5.7	4.9	0.25		*5*
	High NDF		43.2	31.7	16.5	2.510	1.820 ↑		122	103	6.9	5.8	5.0	0.10		

Table 2. (continued).

Breed	Diet	Stage	NDF (% DM)	NFC (% DM)	Starch (% DM)	DMI (kg/d)[a]	Milk (kg/d)	FCM (g/d)[b]	Fat (g/d)	Protein (g/d)	Fat (%)	Protein (%)	Lactose (%)	BCS[c] var.	BW var. (g/d)[d]	Ref.[e]
Assaf	Control diet, starchy pellets (C)	from 60 to 120 DIM	29.3	44.3	35.7	2.44	2.110		110	110	5.16	5.21↑	4.98		19	*6*
	Experimental diet, soy hulls pellets		44.0	29.3	21.4	2.71 ↑	2.410 ↑		150 ↑	110	6.05 ↑	4.67	4.85		25	
Sarda	High NFC	mid-lactation	37.6	35.1	25.2	2.602	1.813		90	85	5.06	4.68 ↑	5.11		173	*7*
	Low NFC		50.6	23.5	7.9	2.946 ↑	2.110 ↑		109 ↑	94 ↑	5.19	4.51	5.03		163	

* days in milk; ** include sugars; [a] Dry matter intake; [b] fat-corrected milk yield; [c] body condition score variation; [d] body weight variation; [e] references;
[1] Cavani et al., 1990; [2] Cannas et al., 1998; [3] Zervas et al., 1998; [4] Bovera et al., 2004; [5] Cannas et al., 2004; [6] Zenou and Miron, 2005; [7] Cannas et al., 2013

4. FIGURES

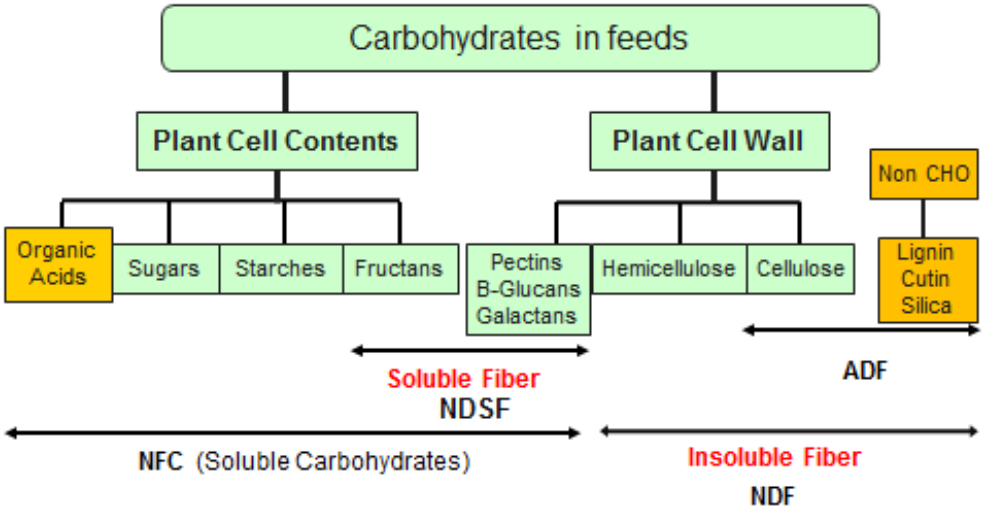

Figure 1. Carbohydrates (in green), associated compounds (in orange) and their cumulative fractions in feedstuffs. NDF: neutral detergent fiber, ADF: acid detergent fiber, NDSF: neutral detergent soluble fiber (includes all non-starch polysaccharides not present in NDF), NFC: non fiber carbohydrates. Adapted from Hall (2000)

CHAPTER 2

High and low-starch diets differently affect milk production and variations in body reserves in dairy goats and ewes during mid-lactation

ABSTRACT

The effect of diets with different levels of non-fiber carbohydrates (NFC) on milk yield and body reserves was studied in 30 mid-lactating mature Sarda dairy sheep and in 26 mid-lactating mature Saanen dairy goats from 92 ± 11 days in milk (DIM; mean ± standard deviation) to 139 ± 11 DIM. Each species was allocated to two dietary treatments: high-starch (HS: 20.0% starch, 36.7% neutral detergent fiber (NDF) and 15.5% crude protein (CP), on DM basis) and low-starch (LS: 7.8% starch, 48.8% NDF and 15.6% CP, on DM basis) diets. The diets contained 28% of dehydrated chopped alfalfa, 3.9% of mature ryegrass hay, and 65.7% of experimental pellets (high-starch or low-starch pellet; % as fed) fed *ad libitum*. In addition, 100 g/day of whole corn grain were administered during the two daily machine milkings. Milk production was measured and milk samples were collected at the daily milkings once a week in order to determine fat, protein, lactose, somatic cell count (SCC) and urea content. Body weight (BW) and body condition score (BCS) estimated at the lumbar region were determined every two weeks. Data were analyzed by using the PROC MIXED procedure of SAS with repeated measurements (SAS Version 9.0). In the mid-lactating goats, the HS diet increased milk production (mean ± SEM; 2.66 vs. 2.53 kg/d ± 0.04; $P<0.05$), fat-corrected milk yield (FCM) (2.65 vs. 2.53 kg/d ± 0.05; $P<0.05$), net energy for lactation (NE_L) (1.88 vs. 1.80 Mcal of NE/d ± 0.03; $P<0.05$), milk protein yield (83.15 vs. 79.84 g/d ± 1.45; $P<0.05$), milk lactose concentration (4.42 vs. 4.39% ± 0.01; $P<0.05$), milk lactose yield (117.45 vs. 111.29 g/d ± 2.16; $P<0.01$), and milk urea (43.95 vs. 41.69 mg/dl ± 0.82; $P<0.05$), whereas it reduced milk fat concentration (3.50 vs. 3.64% ± 0.06; $P<0.05$) compared to the LS diet. Milk fat yield, milk protein concentration, somatic cell count (SCC), body weight (BW) and body condition score (BCS) did not differ between the two diets in goats. In the mid-lactating sheep, the LS diet increased FCM (1.47 vs. 1.36 kg/d ± 0.04; $P<0.01$), NE_L (1.53 vs. 1.41 Mcal of NE/d ± 0.04; $P<0.01$), milk fat concentration (6.68 vs. 6.41% ± 0.07; $P<0.01$), milk fat yield (96.61 vs. 87.88 g/d ± 2.58; $P<0.01$), milk protein concentration (5.16 vs. 5.06% ± 0.04; $P<0.05$) and milk protein yield (74.29 vs. 69.21 g/d ± 2.02; $P<0.05$) compared to the HS diet. Milk production, milk lactose concentration, milk lactose yield, SCC and milk urea did not differ between diets in sheep. In this species, BW did not differ between the two diets, whereas BCS was greater in HS than in LS groups (3.46 vs. 3.33 ± 0.05; $P<0.05$). In conclusion, the high-starch diet fed during mid-lactation was positively associated with milk production in dairy goats and body reserves in dairy sheep.

Key words: starch, NDF, milk production, body reserves, ewes, goats

1. INTRODUCTION

Maintaining an optimal starch to neutral detergent fiber (**NDF**) ratio in the diets is important for high milk yield in dairy ruminants (Nocek and Russell, 1988; Cannas et al., 2002). Starch and, more in general, non-fiber carbohydrates (**NFC**, *i.e*. an estimate of the sum of simple sugars, starch and pectins) are the most important energy substrates to sustain both gluconeogenesis in the liver from propionate, a volatile fatty acid produced in the rumen, and microbial activity at rumen level (Nocek and Russell, 1988). Indeed, as starch degradation in the rumen increases, the amount of microbial N that flows into the duodenum increases, as long as pH does not become too low due to propionate accumulation (Firkins et al., 2001; Dann et al., 2014). Fiber, instead, is necessary for proper rumen function and for pH regulation, because it stimulates rumen contractions, rumination activity and saliva production, which acts as a buffer against rumen pH decrease. In addition, rumen fiber fermentation produces large amounts of acetate, a weaker acid compared to propionate, thus limiting rumen pH reduction. Acetate is also a precursor of body and milk fatty acids and a metabolic energy fuel.

Unfortunately, optimal dietary starch, NFC and NDF reference values during the lactation of small ruminants have not been fully developed, even though during the lactation there might be a variation in the optimal type and amount of carbohydrates to be used. As pointed out by Peel and Bauman (1987), homeorhetic regulation occurs during lactation. In early lactation ruminants are stimulated to use glucose for milk synthesis, due to the dominance of the growth hormone (**GH**) control in energy partitioning, whereas later on insulin becomes more important, favoring the utilization of metabolic fuels for body reserves accumulation rather than milk production. Based on these assumptions, the utilization of diets rich in starch in the second half of lactation would favor body reserves accumulation by increasing hematic glucose and insulin action. By contrast, diets rich in digestible fiber should be able to provide energy without stimulating insulin action too much. This phenomenon might not be so important in dairy cows, whose intense genetic selection have increased the persistency of GH action in favor of milk synthesis (Peel and Bauman,1987), whereas dairy sheep and dairy goats might be more affected by high-starch concentration in mid-late

lactation. The reason for this is that both species, especially sheep, have been subjected to a much less intense genetic selection compared to cows, and are thus likely more sensitive to the insulin effects in favor of glucose utilization for body reserves accumulation (Cannas et al., 2002). Thus, it would be important to better understand the implications of the homeorhetic control of lactation in dairy small ruminants and to test if NFC, especially starch, can impact milk production in mid-lactation, when ewes are likely more prone to insulin action.

In dairy sheep in early lactation, when the energy balance of the ewes is often negative, diets rich in starch (>25-30%, on DM basis) almost always reduce energy deficit and increase milk production, as reviewed by Cannas et al. (2002). Similarly, in highly productive dairy goats, which normally use 60-85% of total glucose for milk synthesis (Annison and Linzell, 1964; Hart, 1983), only high-starch diets can guarantee it (Hart, 1983). Similarly, high-starch diets have positive effects on milk production and milk protein, fat and lactose yield in dairy cows, especially in early lactation (Chagas et al., 2009). Conversely, several experiments conducted on ewes during mid and late-lactation, when their energy balance is certainly positive, showed that diets rich in highly digestible fiber (e.g. with high content of beet pulps or soybean hulls) increased milk production, whereas high-starch diets stimulated fattening (Cavani et al., 1990; Cannas et al., 2002; Cannas et al., 2004; Bovera et al., 2004, Zenou and Miron, 2005; Cannas et al., 2013).

A positive effect of digestible fiber in mid and late lactation has not been observed in goats, which normally respond positively to high-starch diets also in this lactation stage, as observed by Rapetti et al. (2005) and Cannas et al. (2007) in goats fed total mixed rations and by Fedele et al. (2002) in goats fed free-choice diets. This is in full contrast to what observed in sheep in the same lactation stage. These differences might be due to species differences, innate or due to genetic selection, in the concentration of hormones (e.g. GH and insulin) or in the responsiveness of the tissues involved in energy partitioning and blood glucose utilization in mid-late lactation. Other explanations could be differences in acetate and glucose requirements for milk synthesis, because of the different fat to lactose ratio in the milk of the two species (Cannas et al., 2002) or the better ability of goats to divide starch-rich diets into small meals compared to sheep, as

suggested by Abijaoudé et al. (2000), which would therefore diminish insulin hikes. However, these hypotheses are merely speculative because, to our knowledge, no studies have compared the responses of sheep and goats to different levels of starch and digestible fiber fed in the same experimental conditions and same lactation stage.

Thus, the objectives of this study were to test if dietary NFC content, especially starch, can impact milk production in mid-lactation, when animals are more prone to insulin action, and if sheep and goats are differently affected by dietary starch and, consequently, fiber concentration.

2. MATERIALS AND METHODS

The experiment was conducted at the experimental farm of AGRIS, Department of Research on Animal Production, located in Olmedo, in the north-west of Sardinia, Italy.

2.1 Experimental procedure: animals and diets

Thirty mid-lactating mature Sarda dairy sheep and 26 mid-lactating mature Saanen dairy goats were monitored from 92 ± 11 days in milk (DIM; mean ± standard deviation) to 139 ± 11 DIM. Animals were kept in a closed barn, in 4 large pens (2 per species, 68.4 m^2/pen), each pen had access to an external paddock (54 m^2/paddock). Each pen had a water trough with fresh and clean water that allowed adequate drinking space for all animals.

The animals were selected from a larger group fed a high-starch diet, very similar to the experimental diet since parturition. Before selection, all animals had been treated against gastrointestinal parasites (albendazole; Valbazen, Pfizer Italia, Rome).

During a 30-day adaptation period, all animals were fed the same high-starch diet used later in the experimental period. At the end of the adaptation period, the animals were allocated to two dietary groups: high-starch (**HS**; 15 sheep: 88 ± 15 DIM; 13 goats: 94 ± 9 DIM; mean ± standard deviation) and low-starch (**LS**; 15 sheep: 87 ± 12 DIM; 13 goats: 97 ± 5 DIM) group. The subgroups were balanced within species to have the same average body condition score (**BCS**; goats: 2.84 vs. 2.79; sheep: 3.39 vs. 3.33; HS

vs. LS diet; respectively) and milk production (goats: 3.13 vs. 3.12 kg/d; sheep: 1.76 vs. 1.78 kg/d; HS vs. LS diet; respectively).

All the animals were fed a diet (Table 1) containing 29.0% of dehydrated chopped alfalfa, 4.0% of mature ryegrass hay, and 67.0% of experimental pellets (as fed basis). The experimental pellets differed depending on the group as follows: i) for the HS group a high-starch pellet, with 28.1% starch and 30.7% NDF, and ii) for the LS group a low-starch pellet, with 10.0% of starch and 48.8% of NDF (on DM basis; Table 2). The pellets differed mainly because most of the corn meal and all the barley meal of the high-starch pellet were replaced with soybean hulls, a high source of highly-digestible fiber, in the low-starch pellet (Table 2). In addition, 100 g/day per head of whole corn grain (69.6% starch, 16.7% NDF, 8.0% CP, on DM; Table 1) was offered during the two daily machine milkings (7:00 and 15:00).

The two diets were iso-proteic (15.5% and 15.6% of crude protein (CP) for HS and LS diets, respectively), whereas the carbohydrate concentration differed between the two groups as follows: 36.7% NDF, 35.4% NFC and 20.0% starch for the HS diet; and 48.8% NDF, 23.0% NFC and 7.8% starch for the LS diet, on DM basis; Table 1). The high-starch pellet showed a higher NFC and starch concentration and a lower NDF than the low-starch pellet (NFC: 42.6% vs. 24.0%; starch: 28.1 vs. 10.0%, NDF: 30.7% vs. 48.8%, on DM basis) (Table 2).

The diets were group fed *ad libitum* and were supplied twice daily (morning and afternoon) immediately after each milking. The pellets and the dehydrated chopped alfalfa were mixed together and supplied in a large manger, to which all animals had free access, whereas the mature ryegrass hay was supplied at the same time but in another manger. Orts were quantified daily to guarantee at least 10% excess in the diet supplied compared to the actual intake every day.

2.2 Measurements and sampling

Orts derived from the pellet-alfalfa mix or ryegrass hay were collected and weighted separately every day. The diet actually eaten was then calculated as the weekly difference between the diet supplied and the orts. Samples of feeds and orts were

collected weekly for subsequent chemical analysis. Once a week, at the morning and afternoon milkings, individual sheep and goats milk production was measured and individual milk samples were collected and immediately stored at 4°C until analysis.
Individual BCS and body weight (**BW**) were assessed every two weeks. BCS was estimated by 3 trained investigators who assessed the degree of fattening around the lumbar region using a 0 to 5 point scale, with minimum intervals of a quarter of a point, according to the Russel et al. (1969) method. BW was measured by using an electronic scale before the morning meal.

2.3 Chemical analyses

The samples of feeds and orts were ground with a Hammer mill by using a 1 mm screen and then analyzed for DM after drying at 105 °C, CP (Kjeldahl), NDF, ADF, ADL (as reported by Van Soest et al. (1991), including termostable α-amilase), ether extract (Soxlet), starch (polarimetric method; Commission Directive 1999/79/EC of 27 July 1999) and ash. The dietary ingredients and the orts rich in starch were pre-treated overnight with 8 M urea. The NDF and ADF values were quantified on an ash-free basis. The NFC were calculated with following formula: NFC = 100 – CP – NDF – ash – ether extract.

The morning and afternoon milk samples were analyzed separately for fat, protein (N × 6.38), lactose (infrared method; Milkoscan 4000, Foss Eletric, Hillerød, Denmark), urea content (enzymatic-colorimetric method based on Berthelot reaction; Chemspec 150, Bentley Instruments Inc., Chaska, MN, USA) and somatic cell count (SCC, flow-cytometry method; Fossomatic 5000, Foss Electric, Hillerød, Denmark).

2.4 Calculations

Daily milk production and composition were obtained through the weighted mean between the morning and afternoon production and composition data. Fat-corrected milk yield (**FCM**) was calculated separately for the two species. For ewes, milk production was normalized at 6.5% fat as $FCM_{(6.5\%)} = 0.37 \times$ milk yield (kg/d) + 9.7 ×

milk fat (%) × milk yield (kg/d), according to the equation developed by Pulina et al. (1989). For goats, milk production was normalized at 3.5% fat as $FCM_{(3.5\%)}$ = 0.63 × milk yield (kg/d) + 10.5 × milk fat (%) × milk yield (kg/d), according to Pulina et al. (1992).

Milk energy content was calculated separately for the two species. Net energy for lactation (NE_L) (Mcal of NE/d) was calculated as NE_L = (251.73 + 89.64 × PQ + 37.85 × (PP/0.95)) × Yn/1000 for ewes, and NE_L = (289.72 + 71.93 × PQ + 48.28 × (PP/0.92)) × Yn/1000 for goats, according to Tedeschi et al. (2010). In particular, Yn is measured milk yield at a particular day of lactation (kg/d), PQ is measured milk fat at a particular day of lactation (%), PP is measured true milk protein for a particular day of lactation (%).

2.5 Statistical analysis

Data on milk production and composition, BW and BCS were analyzed by the PROC MIXED procedure of SAS (Version 9.0, SAS Institute Inc., Cary, NC, USA) with repeated measurements. In particular, a mixed model was used to test the differences between the diets as reported in the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma + \pi_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the general mean, α_i is the effect of diet (i=HS, LS), β_j is the effect of period (j=1-6 Milk; j=1-4 BCS and BW), $\alpha\beta_{ij}$ is the diet × period interaction (i=HS, LS; j=1-6 Milk; j= 1-4 BCS and BW), γ is the random effect of animal, π_{ij} is the covariate and ε_{ij} is the residual error. Data of the pre-experimental period were included in the model as covariate. SCC was log transformed. Data were expressed as mean ± SEM. Means were separated using Tukey's test. The accepted level of significance was $P<0.05$.

3. RESULTS

3.1 Feed composition and intake

Based on the chemical analysis of the two experimental diets (Table 1), the HS diet had a higher NFC and starch concentration than the LS diet. The LS diet had a higher NDF, ADF, ADL and ash concentration compared with the HS diet (Table 1).

The concentration and intake of the diets actually eaten by the animals (Table 3) was based on group average values, therefore these data were not submitted to statistical analysis. Overall, the differences in chemical composition between the diet supplied (Table 1) and that eaten (Table 3) were limited. Due to the limited dietary selection observed during the trial, the concentration of the diets actually eaten were very similar between ewes and goats within the same dietary treatment (HS vs. LS) (Table 3).

In the goats, the average dietary DM and CP intake during the trial were almost identical for the two diets (Table 3), whereas, as expected, the intake of NDF was much higher for the LS than for the HS group (1.33 vs. 1.01 kg/d). The contrary occurred for the intake of NFC and starch, as expected. As a result of the combined effect of the similarity in DM intake and the difference in dietary energy concentration, the NEL intake of the HS group was +6.6% higher than that of the LS group. The evolution of feed intake was similar between HS and LS goats (Figure 1).

In the ewes, the dietary DM intake was numerically slightly higher (+3.8%) in the LS than in the HS group (Table 3). Dietary CP intake was very similar between groups, whereas the NDF intake was much higher in the LS than in the HS group (1.32 vs. 0.93 kg/d), as planned when defining the experimental design. The contrary occurred for the intake of NFC and starch, as expected. As a result of the combined effect of the slight difference in DM intake and the difference in dietary energy concentration of the diets of the ewes, the dietary NEL intake of the HS group was only slightly higher (+2.4%) than that of the LS group.

Comparing the two species, the DM intake was higher in goats than sheep for the HS diet and the same for the LS diet. As a result, the daily intake of nutrients was similar between the two species when compared within dietary treatment.The evolution of feed

intake was similar between HS and LS sheep during the first part of the experiment and changed in the last part of the experiment, being higher in the LS than in the HS sheep (Figure 2)

3.2 Milk production and composition

Effect of diet

In Saanen goats, milk yield decreased in both dietary groups after the preliminary period (Figure 3; Table 4). During the experimental period, milk yield was higher in the HS than in the LS group (2.66 vs. 2.53 kg/d ± 0.04 (mean ± SEM); P=0.011) (Table 4). FCM and NE_L were also higher in the HS than in the LS group (FCM: 2.65 vs. 2.53 kg/d ± 0.05, P=0.019; NE_L: 1.88 vs. 1.80 Mcal of NE/d ± 0.03, P=0.025). Milk fat concentration was lower in HS fed goats than in LS fed goats (3.50 vs. 3.64%; P = 0.031), whereas milk fat yield did not differ between the two diets (92.8 vs. 89.6 g/d ± 2.1 for HS and LS diets, respectively) (Table 4). Milk protein concentration did not differ between HS and LS diet (3.14 vs. 3.17 % ± 0.02 for HS and LS, respectively), whereas milk protein yield was greater in goats fed the HS diet than goats fed the LS diet (83.2 vs. 79.8 g/d ± 1.45; P = 0.033) (Table 4). Milk lactose concentration (4.42 vs. 4.39% ± 0.01; P=0.038) and milk lactose yield (117.5 vs. 111.3 g/d ± 2.16; P=0.009) were higher in HS fed goats and in LS fed goats (Table 4). Milk urea content increased with the HS diet compared to the LS diet (43.95 vs. 41.69 mg/dl ± 0.82; P= 0.012), whereas SCC did not vary with diet (Table 4).

In Sarda ewes, milk yield decreased in both groups after the preliminary period (Figure 4; Table 5). During the experimental period, sheep milk yield was not statistically different between the two diets but was numerically greater in the LS than in the HS group (1.44 vs. 1.38 kg/d ± 0.04) (Table 5). FCM and NE_L were higher in the LS than in the HS group (FCM: 1.47 vs. 1.36 kg/d ± 0.04; P=0.008; NE_L: 1.53 vs. 1.41 Mcal of NE/d ± 0.04; P=0.008). The ewes fed the LS diet had a higher milk fat concentration (6.68 vs. 6.41% ± 0.07; P=0.001), milk fat yield (96.6 vs. 87.9 g/d ± 2.6; P=0.002), milk protein concentration (5.16 vs. 5.06% ± 0.04; P=0.014) and milk protein yield (74.3 vs. 69.2 g/d ± 2.02; P=0.018) compared to the ewes fed the HS diet (Table 5). Milk lactose

concentration and milk lactose yield did not differ between the two diets in sheep but were numerically greater in the LS than in the HS group (milk lactose concentration: 4.52 vs. 4.46 % ± 0.04; milk lactose yield: 67.2 vs. 63.5 g/d ± 1.95) (Table 5). The SCC and milk urea did not differ between the two groups (SCC: 2.88 vs. 2.96 log SCC ± 0.06, urea: 37.4 vs. 38.3 mg/dl ± 0.50 for the LS and HS diets, respectively).

Effect of period

In goats, the effect of period was statistically significant for NE_L (P=0.041), milk fat concentration (P<0.0001), milk fat yield (P=0.0009), milk protein concentration (P=0.048), milk lactose concentration (P=0.024), SCC (P=0.002) and milk urea concentration (P<0.0001), whereas it was not significant for milk yield, FCM, milk protein yield and milk lactose yield (Table 4).

In sheep, the effect of period was statistically significant for milk fat (P=0.047), milk protein (P=0.017), and milk urea (P<0.0001) concentration, whereas it was not significant for milk yield, FCM, NE_L, milk fat and protein yield, milk lactose concentration and yield, and SCC (Table 5).

Effect of diet x period interaction

In goats, the diet × period interaction was not significant for any of the milk parameters considered (Table 4). In sheep, the diet × period interaction influenced significantly milk urea (P<0.0001) and milk protein (P=0.05) concentration (Table 5).

Effect of the covariate

In both species, the covariate was statistically significant (P<0.0001) for all parameters considered (Table 4), except for sheep milk lactose yield (Table 5).

3.3 Evolution of body reserves

Effect of diet

In Saanen goats, BW was not affected by the dietary treatments (60.02 vs. 60.12 kg ± 0.38 for HS and LS diets, respectively; Table 6). In HS fed goats, BW decreased from

the preliminary period to the first experimental period but tended to increase afterwards. Differently, in LS fed goats, BW showed a decrease from the preliminary period to the second experimental period, followed by an increase in the third period, and a decrease in the last period (Figure 5; Table 6). In goats, BCS was not affected by the dietary treatments either (2.77 vs. 2.74 ± 0.03 for HS and LS, respectively; Table 6), but it was numerically greater in goats fed the HS diet over time (Figure 6), with a different trend compared to that observed for BW (Figure 5).

In Sarda ewes, BW did not differ between the two diets (Table 7) and tended to have the same trend for both groups (Figure 7). By contrast, BCS was greater in HS fed ewes than in LS fed ewes (3.46 vs. 3.33 ± 0.05; P=0.013; Table 7). After an initial decrease from the preliminary period to the first experimental period, BCS showed an increase over time, which was more pronounced for the HS group than for the LS group (Figure 8).

Effect of period and diet x period interaction

In goats, the effect of period was significant for BW (P=0.002) and tended to be significant for BCS (P=0.053) (Table 6). In sheep, the effect of period was significant for both BW (P<0.0001) and BCS (P=0.0002) (Table 7).

The diet × period interaction was not significant for BW and BCS in goats (Table 6) and sheep (Table 7).

Effect of the covariate

The covariate was significant (P<0.0001) for both BW and BCS in goats (Table 6) and sheep (Table 7).

4. DISCUSSION

4.1 Feed intake

The intake of the goats was very similar for the two diets (Figure 1), as previously observed in mid-lactating Murciano-Granadina goats fed high-starch (starch 21.9% and

NDF 40.6%, on DM basis) or low-starch (starch 7.0% and NDF 46.5%, on DM basis) diets (Ibáñez et al., 2015). In contrast, Cannas et al. (2007) observed an 8% higher intake in low-starch-high digestible fiber diets (NDF 44.7% and NFC 29.3%, on DM basis) compared to high-starch diets (NDF 36.9% and NFC 36.0%, on DM basis). The reasons for these contrasting results are not clear.

In sheep, the numerically higher DMI in the LS compared to the HS group (Figure 2) is in accordance to previous studies on lactating ewes in mid-lactation comparing low-NFC diets and high-NFC diets, in which cereal grains were replaced with highly-digestible fiber sources (Cannas et al., 1998; Zenou and Miron, 2005; Cannas et al., 2013). As in the studies just mentioned, the combined effect of the difference in DMI (higher for the LS diets) and energy concentration (higher for the HS diets) between groups caused a very similar daily energy intake between the two dietary treatments (Table 3). This is probably due to the fact that intake was likely regulated by energy requirements and not by rumen fill. Indeed, the ewes were able to eat higher amounts of the LS diet compared to HS diet to compensate for the lower energy concentration of LS diet. This was possible because soybean hulls were used to replace cereal grains in the LS diets. This ingredient has a small particle size and thus does not saturate the physical capacity of the rumen, as previously suggested (Cannas et al., 2002; Cannas et al., 2013). Other possible causes of the difference in intake observed in this experiment and in the literature when high-starch and highly-digestible fiber diets are compared could be the high production of propionic acid derived from high-starch diets, which were associated with increased satiety (Roche et al., 2008). Similar patterns were also observed in dairy cows (Beckman and Weiss, 2005).

4.2 Milk production

The fact that the HS diet had a positive effect on milk production, FCM, and NE_L content in goats but a negative effect on FCM, NE_L, and, numerically, on milk yield in sheep evidenced that the two species under study responded differently to the dietary treatments applied during mid-lactation.

The positive effect of the HS diet on mid-lactating Saanen goats found in our trial is in accordance with previous studies comparing high and low NDF diets in mid-lactating Sarda goats (Cannas et al., 2007), and high and low-starch diets (high-starch 21.9%, NFC 28.7%, NDF 40.6%; low-starch: starch 7.0%, NFC 28.7%, NDF 46.5%, on DM basis) in mid-lactating Muciano-Granadina goats (Ibáñez et al., 2015) as well as studies on dairy goats fed high concentrate diets (70% of the diet; NDF 36%; starch 9.7%, on DM basis) (Serment et al., 2011) and dairy cows fed high-starch and sugar diets (Piccioli-Cappelli et al., 2014). However, other authors observed no differences due to dietary starch and fiber level (Kawas et al., 1991) or a curvilinear pattern, such as those reported by Goetsch et al. (2001) on late-lactating Alpine goats, in which milk production increased with diets containing 50% of concentrate (CP: 16.7%, NDF: 40.9%, on DM) but decreased when the amount of concentrate in the diet increased up to 65% (CP: 16.4%, NDF: 35.1%, on DM).

The positive effect of the LS on mid-lactating sheep observed in our experiment is in accordance with previous studies in which milk yield was higher in ewes fed low NFC-high digestible fiber diets compared to ewes fed high NFC diets (Cavani et al., 1990; Cannas et al., 1998; Bovera et al., 2004; Zenou and Miron, 2005; Cannas et al., 2013). Similarly, other studies reported a negative effect or no effect of high NFC-diets on milk production in ewes in mid-lactation (Molle et al., 1997; Bocquier et al., 2002).

Role of starch and fiber in milk production

The scientific opinion about the positive or negative effects of high-starch diets on ruminants is still controversial. In fact, some authors found a positive effect on milk yield, whereas others reported an increase in body fat deposition.

As reported by Morand-Fehr et al. (2007), high-starch diets increase milk production because their fermentation in the rumen leads to the production of propionic acid, which, due to the glucogenic process that occurs in the liver, causes an increase in the concentration of blood glucose; the latter then flows towards the mammary gland to synthesize lactose and then produce milk. In fact, the key element essential for milk production is blood glucose uptake by the mammary gland because it is the most important precursor of lactose synthesis and is also involved in ATP production and in

protein, lipid and nucleotide synthesis (Zhao and Keating, 2007). The amount of lactose synthesized in the mammary gland is linked to the glucose absorbed by the udder. For this reason, the mammary gland is considered a glucose-dependent organ (Scott et al., 1976). In dairy goats, from 60 to 85% of the glucose used by the animal is directed towards milk synthesis (Annison and Linzell, 1964). The mammary gland uses glucose to produce in sequence lactose, protein and milk fat (Pulina et al., 2005).

Serment et al. (2011) hypothesized that the positive effect of a high concentrate, high-starch diet on milk yield could be linked to the increase in rumen microbial protein that occurs in ruminants fed this type of diet compared to those fed a low concentrate diet. On the other hand, other studies have shown that high-starch diets can have a negative effect on milk production and a positive effect on body fat deposition linked to an increase in blood insulin (Grum et al., 1996; van Knegsel et al., 2007a; van Knegsel et al., 2007b; Boerman et al., 2015) or to the production of biohydrogenation intermediates (Boerman et al., 2015). Goetsch et al. (2001) suggested that with moderate amounts of concentrate, the energy of the diet is used to restore body reserves mobilized in early lactation, whereas with high amounts of concentrates the competition between the udder and body reserves for some nutrients, such as glucose and NEFA, is enhanced (Gaynor et al., 1995). The increase in body fatness in dairy cows fed high-starch diets can be due to the production of biohydrogenation intermediates linked to milk fat depression (Bauman et al., 2011). In particular, it has been shown that trans-10, cis-12 C18:2 is involved in the decrease in gene expression of lipogenic enzymes in the udder and the increase in lipogenic gene expression in the adipose tissue (Harvatine et al., 2009; Boerman et al., 2015). In brief, high-starch diets can change nutrient partitioning from the mammary gland to the adipose tissue, by increasing the production of trans-10, cis-12 C18:2 in the rumen or plasma insulin (Boerman et al., 2015). As a result, the animal tends to become overweight and reduce milk fat production. Certainly, an important factor is the concentration of starch used, because high concentrations, above the optimal level, can cause a drop in rumen pH and negative effects on diet utilization and milk production, whereas optimal concentrations can stimulate milk yield.

A possible hormonal mechanism in regulating the energy partitioning between milk production and body reserve accumulation observed in this experiment will be investigated in Chapter 3 of this dissertation.

4.3 Milk composition

Milk fat

In our experiment, the HS diet reduced milk fat concentration in both species. However, milk fat yield did not differ between the two diets in goats, whereas it was lower for HS diets in sheep. It is commonly known that diets rich in NSC decrease milk fat also in other species such as dairy cows (Petitclerc et al., 2000), whereas diets rich in fiber increase milk fat. This happens because the acetate derived from fiber fermentation is considered one of the most important lipid precursors in ruminant species (Linington et al, 1998) and probably because the high rumen pH associated with fiber fermentation favors bio hydrogenation processes, thus reducing the production of trans fatty acids that might stimulate milk fat depression (Griinari et al., 1997). In fact, there is a positive relationship between NDF and milk fat concentration, as reported in the equation for dairy sheep of Pulina and Rassu (1991), *i.e.* fat (g/100 ml) = 4.59 + 0.005 NDF (g/kg DM); R^2=0.23, and confirmed by the positive correlation of +0.38 between these two parameters reported by Nudda et al. (2004). In lactating cows, Piccioli-Cappelli et al. (2014) found that milk fat was higher with a low-starch and low sugar diet than with a high-starch and high sugar diet administered in late lactation.

In Sarda goats (milk yield: 1336 ± 242 g/d; 5th month of lactation), milk fat concentration did not differ between high-starch and low-starch diets (4.09% and 4.08% milk fat, respectively), whereas milk fat yield was higher for the low-starch compared to the high-starch diet (65.30 vs. 57.60 g/d; P<0.001) (Cannas et al., 2007). In Murciana Granadina goats (106 DIM), low-starch diets increased milk fat concentration compare to high-starch diets (6.4 vs. 5.4% ± 0.17; P=0.01) (Ibáñez et al., 2015), even though this could be partially an effect of the decreased milk production observed with the low-starch diets.

Based on studies in which a higher milk fat concentration occurred in mid lactating ewes fed low NFC diets (Cannas et al., 1998; Zenou and Miron, 2005; Cannas et al. 2013), Cannas et al. (2013) hypothesized that high fiber diets but not high-starch diets increase milk fat due to a high acetate synthesis.

The observed reduction in milk fat concentration in sheep and goats fed the HS diet could also be due to a high level of blood insulin, which stimulates lipoprotein lipase activity in adipose tissues and reduces the partitioning of fatty acids towards the mammary gland (Gaynor et al., 1995; Reynolds et al., 2001), thus decreasing the milk fat yield (Rao et al., 1973; McClymont, 1951). In fact, milk fat concentration and milk fat yield were lower in Holstein cows (109 ± 22; DIM ± standard deviation) fed a high-starch diet (1.68 kg/d and 3.68% of milk fat yield and concentration, respectively) compared to a high fiber and high fat diet (1.81 kg/d and 3.95% of milk fat yield and concentration, respectively) and were negatively correlated with insulin and blood glucose and trans-10, cis-12 C18:2 (Boerman et al., 2015).

Milk protein

In our experiment, the response in terms of milk protein differed between the two species. In goats, the HS diet increased milk protein yield but not milk protein concentration compared to the LS diet, whereas in ewes milk protein concentration and yield were higher in the LS group. These results are in accordance with a previous study conducted on Sarda goats, in which milk protein concentration was higher with a low-starch diet than with a high-starch diet (4.30 vs. 4.20%; $P<0.005$), probably due to the concentration effect, as suggested by the authors, whereas milk protein yield was higher for the high-starch than for the low-starch diet (67.40 vs. 59.50 g/d; $P<0.001$) (Cannas et al., 2007). In Murciana Granadina goats (106 DIM), milk protein did not vary between a high-starch and a low-starch diet (Ibáñez et al., 2015). Our results on sheep are also in contrast with other studies conducted on mid-lactating ewes, where milk protein concentration was higher in the high NFC group than in the low NFC group (4.68 vs. 4.51% ± 0.07; $P< 0.016$), due to the concentration effect caused by the reduced milk yield, whereas milk protein yield was higher in the low NFC group (Cannas et al., 2013).

In dairy cows fed increasing amounts of dietary NSC, milk protein showed a variable trend, but tended to increase (Petitclerc et al., 2000). Some authors suggested that high-energy diets are associated with an increase in sheep milk protein (Bocquier and Caja, 2001) due to the effect of the insulin (Mackle et al., 1999) or to the increase in microbial protein production (Boerman et al., 2015). Boerman et al. (2015) hypothesized that an increase in insulin concentration leads to an increase in milk protein concentration and milk protein yield (McGuire et al., 1995; Griinari et al. 1997; Boerman et al., 2015), probably due to "the activation cascade for the synthesis of proteins" (Winkelman and Overton, 2013). In Holstein cows (109 ± 22; DIM ± standard deviation), milk protein concentration and milk protein yield were higher when fed a high-starch diet (1.44 kg/d; 3.07%) than a high fiber and fat diet (1.34 kg/d; 2.93%), and these two parameters were positively correlated with insulin and blood glucose and trans-10, cis-12 C18:2 (Boerman et al., 2015). Similarly, Piccioli-Cappelli et al. (2014) found that milk protein in dairy cows in late lactation was higher when fed a high-starch plus sugar diet compared to a low-starch plus sugar diet.

Differently, in Saanen goats, milk protein yield and concentration did not differ between a non-forage diet and the control diet (silage-based) from early to mid-lactation (Bava et al., 2001).

Other milk components

In our experiment, the higher milk lactose concentration and yield found in the HS fed goats followed their greater milk yield compared to LS fed goats. These findings were in contrast with previous studies in which milk lactose concentration did not change when goats were fed a high-starch diet (Ibáñez et al., 2015). In sheep, the observed lack of effect of diet on milk lactose concentration and yield is in accordance with a similar finding of Cannas et al. (2013). In dairy cows, milk lactose was higher in late-lactation cows fed high-starch plus sugar than in those fed low-starch plus sugar (Piccioli-Cappelli et al., 2014).

The lack of difference in SCC observed between the two diets in both species is in accordance to what observed in previous studies on dairy goats (Cannas et al., 2007) and dairy sheep (Cannas et al., 2013).

In general milk urea is inversely associated with the energy and starch content of the diet (Giovanetti, 2006). Indeed, in our study there was a slightly higher concentration of milk urea in the goats fed the HS compared to those fed the LS diet. The lack of dietary effect on milk urea concentration observed in sheep is in contrast with what observed in previous studies on mid-lactating ewes, in which high NFC diets reduced milk urea concentration compared to low NFC diets (Cannas et al., 1998; Cannas et al., 2013). This lack of effect in milk urea concentration was probably due to the fact that the average value calculated in our trial was markedly affected by the much higher value observed in the HS group in the first sampling after the application of the experimental diets (Table 5), whereas for most sampling weeks the values of milk urea concentration were lower in the HS group.

4.4 Evolution of body weight and body reserves

In goats, the fact that BW and BCS were unaffected by dietary treatment is in accordance with Cannas et al. (2007). The evolution of BW (Figure 5) and BCS (Figure 6) in goats evidenced an opposite trend. In fact, the LS fed goats were heavier than HS fed goats, although the values were not statistically different, and had a lower (P=0.011) milk yield, whereas the HS fed goats had a higher BCS. The different evolution among body reserves is likely due to the tendency of dairy goats to accumulate abdominal fat (Dønnem et al., 2011), which makes the estimation of body reserves using lumbar BCS more difficult compared to sheep (Colomer-Rocher et al., 1992; Morand-Fehr, 2005).
In sheep, BW did not change between the two diets, whereas BCS was greater in ewes HS than LS. Nevertheless, the fact that both parameters used to estimate body reserves, *i.e.* BW and lumbar BCS, ha a similar evolution over time (Figures 7 and 8) suggests that in this species body reserves are more easily assessed than in goats. It is well known that there is a different fat deposition between sheep and goats. For this reason, some authors proposed different methods to estimate body reserves in goats compared to sheep. For dairy goats, Santucci (1984) proposed the BCS estimation in the sternal region, due to the tendency of this species to accumulate fat as abdominal and visceral

fat (Dønnem et al., 2011). These findings were confirmed by Delfa et al. (1995) in Blanca Celtibérica goats.

As suggested by Boerman et al. (2015) and other authors (van Knegsel et al., 2007a), nutrient partitioning is driven towards fat deposition with high-starch diets and towards milk production with low-starch plus fat diets in dairy cows. The same authors suggested that the use of low-starch plus fat diets in dairy cows could be the solution to reduce overweight during mid-lactation and therefore to maintain milk yield. In general, in mid-late lactation a balanced diet has a stronger and positive effect on body weight than on milk production, which instead tends to decrease or remain unchanged (Bocquier and Caja, 1993).

No differences in BW were observed previously on highly productive (2198 ± 446 ml/d) Sarda sheep in mid-lactation fed high NFC or low NFC diet (Cannas et al., 2013). The increased body reserves observed in the ewes fed the HS diet in our study, as reported in other studies where the same diet increased body weight (van Knegsel et al., 2007a; Boerman et al., 2015), was probably due to an increase in plasma insulin concentration, which stimulates lipogenesis rather than lipolysis (Boerman et al., 2015). Several studies observed a positive effect of a high NFC diet on BW and a decrease or no effect on milk production on ewes in mid-lactation (Molle et al., 1997; Bocquier et al., 2002; Cannas et al., 2013).

5. CONCLUSIONS

In conclusion, sheep and goats in mid-lactation appear to respond differently to different types of carbohydrates (*i.e*. starch vs. digestible fiber) and to have a different nutrient partitioning towards milk or body reserves. In particular, when considering milk production in mid-lactation, dairy goats take advantage of high-starch diets, whereas sheep benefit from highly digestible fiber. Other comparative studies, particularly on metabolism parameters at different lactation stages, are needed to better understand the mechanism behind the partitioning of nutrients in small ruminant species.

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7. TABLES

Table 1. Ingredient and chemical composition of the high-starch (HS) and low-starch (LS) diets supplied during the experiment.

Period	**Mid-lactation**	
Groups/Diet	**HS**	**LS**
Ingredients (% as fed)		
Pellet (high-starch or low-starch)	67.0	67.0
Dehydrated chopped alfalfa	29.0	29.0
Whole corn grain	*	*
Mature ryegrass hay	4.0	4.0
TOTAL	*100.0*	*100.0*
Chemical composition[a]		
DM (% as fed)	89.6	89.1
CP (% DM)	15.5	15.6
Ash (% DM)	11.0	11.2
Ether extract (% DM)	1.4	1.4
NDF (% DM)	36.7	48.8
ADF (% DM)	25.6	35.5
ADL (% DM)	4.7	5.1
NFC (% DM)[b]	35.4	23.0
Starch (% DM)	20.0	7.8

[a] The chemical composition does not include the corn grains supplied at milking; [b] NFC: 100 – CP – NDF – ash – ether extract; * additional supply of whole corn grain: 100 g/d with the following chemical composition: DM 86.5%, as fed; on a DM basis: CP 8.0%, ash 1.43%, fat 2.1%, NDF 16.7%, ADF 4.7%, ADL 0.9%, NFC 71.8%, starch 69.6%

Table 2. Ingredients and chemical composition of the high-starch and low-starch pellets supplied during the experiment.

Period	**Mid-lactation**	
Groups/Pellet	**High-starch**	**Low-starch**
Ingredients (% as fed)		
Dehydrated alfalfa	30.0	30.0
Corn meal	21.1	3.0
Barley meal	13.4	0.0
Wheat bran	10.1	5.0
Soybean hulls	9.0	43.2
Soybean meal 44	5.0	7.4
Sugarcane molasses	4.6	4.6
Sodium bicarbonate	3.0	3.0
Bentonite	2.0	2.0
Magnesium oxide	1.5	1.5
Minerals and vitamins	0.3	0.3
Appetizer	0.03	0.03
TOTAL	*100.0*	*100.0*
Chemical composition		
DM (% as fed)	90.0	89.3
CP (% DM)	14.1	14.2
Ash (% DM)	11.3	11.6
Ether extract (% DM)	1.4	1.4
NDF (% DM)	30.7	48.8
ADF (% DM)	19.9	34.7
ADL (% DM)	2.9	3.6
NFC (%DM)[a]	42.6	24.0
Starch (% DM)	28.1	10.0

[a] NFC: 100 – CP – NDF – ash – ether extract

Table 3. Actual dietary nutrient and energy intake and concentration of the ewes and goats group fed high-starch (HS) or low-starch (LS) diets. Values corrected for amount and composition of orts. The energy concentration values were those found in the digestibility trial (Chapter 4).

Species	Diet	Diet (kg/d of DM)			Ash	OM	CP	NDF	ADF	ADL	NFC	Starch	NEL[a]
		Supplied	Orts	Eaten									
						Intake (kg/d)							(Mcal NE/d)
Goats	HS	2.97	0.27	2.70	0.29	2.41	0.41	1.01	0.71	0.13	0.94	0.53	4.02
Goats	LS	2.90	0.20	2.69	0.30	2.39	0.42	1.33	0.96	0.14	0.61	0.21	3.77
Sheep	HS	2.84	0.24	2.60	0.28	2.31	0.39	0.93	0.64	0.11	0.95	0.52	3.87
Sheep	LS	2.95	0.25	2.70	0.30	2.40	0.41	1.32	0.96	0.14	0.62	0.21	3.78
						Concentration (% of DM)							(Mcal NE/kg)
Goats	HS	-	-	-	10.9	89.1	15.2	37.5	26.3	4.8	35.0	19.8	1.49
Goats	LS	-	-	-	11.1	88.9	15.5	49.2	35.7	5.2	22.7	7.8	1.40
Sheep	HS	-	-	-	11.0	89.0	15.2	35.7	24.7	4.4	36.7	20.1	1.49
Sheep	LS	-	-	-	11.2	88.8	15.3	49.1	35.7	5.0	23.1	7.9	1.40

[a] Net energy of lactation

Table 4. Evolution of milk yield, fat-corrected milk yield (FCM), net energy of lactation (NE_L), fat, protein, lactose, somatic cell cont (SCC) and urea in goats fed high-starch (HS) and low-starch (LS) diets in mid-lactation.

	Diet	**Prelimin.**	**Period**						**Mean**	**SEM**[g]	***P* level**			
		Period	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]			**D**[h]	**P**[i]	**D×P**[l]	**π**[m]
Milk yield (kg/d)	HS	3.13	2.75	2.73	2.64	2.64	2.49	2.69	2.66	0.04	0.0114	N.S.[n]	N.S.	<0.0001
	LS	3.12	2.55	2.52	2.49	2.57	2.46	2.60	2.53					
FCM (kg/d)	HS	3.15	2.80	2.69	2.65	2.60	2.49	2.69	2.65	0.05	0.0189	N.S.	N.S.	<0.0001
	LS	3.23	2.67	2.53	2.50	2.48	2.46	2.56	2.53					
NE_L (Mcal NE/d)	HS	2.24	1.99	1.90	1.87	1.84	1.76	1.91	1.88	0.03	0.0250	0.0412	N.S.	<0.0001
	LS	2.30	1.89	1.79	1.77	1.76	1.74	1.81	1.80					
Fat (%)	HS	3.61	3.71	3.37	3.50	3.37	3.52	3.52	3.50	0.06	0.0313	<0.0001	N.S.	<0.0001
	LS	3.86	4.09	3.66	3.66	3. 32	3.65	3.47	3.64					
Fat (g/d)	HS	112.50	101.35	91.30	93.00	88.98	87.68	94.53	92.81	2.14	N.S.	0.0009	N.S.	<0.0001
	LS	120.96	101.96	89.69	88.87	82.30	87.04	87.57	89.57					
Protein (%)	HS	3.22	3.22	3.13	3.13	3.08	3.11	3.17	3.14	0.02	N.S.	0.0477	N.S.	<0.0001
	LS	3.19	3.21	3.16	3.17	3.17	3.14	3.16	3.17					
Protein (g/d)	HS	99.37	88.06	85.04	82.56	81.28	77.11	84.86	83.15	1.45	0.0326	N.S.	N.S.	<0.0001
	LS	99.67	81.17	79.42	78.72	81.20	76.81	81.74	79.84					

Table 4. (continued).

	Diet	Prelimin. Period	Period						Mean	SEM[g]	*P* level			
			1[a]	2[b]	3[c]	4[d]	5[e]	6[f]			D[h]	P[i]	D×P[l]	π[m]
Lactose (%)	HS	4.54	4.48	4.43	4.39	4.40	4.41	4.42	4.42	0.01	0.0379	0.0236	N.S.[n]	<0.0001
	LS	4.55	4.43	4.39	4.33	4.40	4.38	4.40	4.39					
Lactose (g/d)	HS	142.34	122.88	120.99	116.11	116.53	109.64	118.54	117.5	2.16	0.0091	N.S.	N.S.	<0.0001
	LS	141.27	112.96	110.91	108.06	113.22	107.87	114.69	111.3					
SCC (log)	HS	2.99	3.15	2.83	3.02	2.97	3.01	2.86	2.97	0.05	N.S.	0.0021	N.S.	<0.0001
	LS	2.88	3.12	2.86	3.08	2.95	2.91	2.83	2.96					
Urea (mg/dl)	HS	39.89	48.28	45.71	46.72	39.85	44.10	39.06	43.95	0.82	0.0117	<0.0001	N.S.	<0.0001
	LS	43.52	41.86	42.83	45.03	39.43	42.51	38.45	41.69					

[a] 29 April; [b] 4 May; [c] 11 May; [d] 18 May; [e] 21 May; [f] 25 May; [g] standard error of the mean; [h] effect of diet; [i] effect of period; [l] diet × period interaction; [m] effect of covariate; [n] P>0.05

Table 5. Evolution of milk yield, fat-corrected milk yield (FCM), net energy for lactation (NE_L), fat, protein, lactose, somatic cell cont (SCC) and urea in sheep fed high-starch (HS) and low-starch (LS) diets in mid-lactation.

	Diet	**Prelimin.**	**Period**						**Mean**	**SEM**[g]	***P* level**			
		Period	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]			**D**[h]	**P**[i]	**D×P**[l]	**π**[m]
Milk yield (kg/d)	HS	1.76	1.45	1.41	1.38	1.32	1.35	1.37	1.38	0.04	N.S.[n]	N.S.	N.S.	<0.0001
	LS	1.78	1.54	1.48	1.41	1.42	1.41	1.40	1.44					
FCM (kg/d)	HS	1.72	1.40	1.39	1.38	1.30	1.33	1.38	1.36	0.04	0.0080	N.S.	N.S.	<0.0001
	LS	1.70	1.56	1.49	1.45	1.42	1.47	1.46	1.47					
NE_L (Mcal NE/d)	HS	1.70	1.45	1.45	1.43	1.34	1.38	1.42	1.41	0.04	0.0082	N.S.	N.S.	<0.0001
	LS	1.68	1.60	1.54	1.51	1.47	1.53	1.51	1.53					
Fat (%)	HS	5.80	6.18	6.30	6.51	6.51	6.44	6.55	6.41	0.07	0.0014	0.0470	N.S.	<0.0001
	LS	5.71	6.60	6.60	6.79	6.37	6.83	6.87	6.68					
Fat (g/d)	HS	102.00	89.32	89.03	89.07	84.17	86.08	89.63	87.88	2.58	0.0022	N.S.	N.S.	<0.0001
	LS	99.27	101.71	96.84	95.84	91.43	97.47	96.39	96.61					
Protein (%)	HS	5.01	5.02	5.16	5.14	5.04	5.02	4.99	5.06	0.04	0.0138	0.0173	0.0502	<0.0001
	LS	4.90	4.91	5.13	5.26	5.20	5.23	5.24	5.16					
Protein (g/d)	HS	86.47	71.87	72.65	70.00	65.43	67.16	68.16	69.21	2.02	0.0181	N.S.	N.S.	<0.0001
	LS	86.51	75.09	75.02	74.00	73.76	74.13	73.75	74.29					

Table 5. (continued).

	Diet	Prelimin. Period	Period						Mean	SEM[g]	*P* level			
			1[a]	2[b]	3[c]	4[d]	5[e]	6[f]			D[h]	P[i]	D×P[l]	π[m]
Lactose (%)	HS	4.61	4.49	4.54	4.45	4.35	4.46	4.48	4.46	0.04	N.S.[n]	N.S.	N.S.	<0.0001
	LS	4.68	4.55	4.46	4.52	4.53	4.52	4.52	4.52					
Lactose (g/d)	HS	82.15	66.41	66.07	63.39	59.30	61.92	63.68	63.46	1.95	N.S.	N.S.	N.S.	N.S.
	LS	84.71	71.69	68.27	65.55	65.92	66.01	65.29	67.18					
SCC (log)	HS	3.06	3.07	3.02	3.12	2.84	2.80	2.90	2.96	0.06	N.S.	N.S.	N.S.	<0.0001
	LS	2.98	2.91	2.86	2.89	2.75	3.00	2.91	2.88					
Urea (mg/dl)	HS	38.94	45.52	38.33	39.34	36.15	36.71	33.93	38.33	0.50	N.S.	<0.0001	<0.0001	<0.0001
	LS	39.18	36.75	39.23	38.06	36.91	38.48	35.07	37.42					

[a] 29 April; [b] 4 May; [c] 11 May; [d] 18 May; [e] 21 May; [f] 25 May; [g] standard error of the mean; [h] effect of diet; [i] effect of period; [l] diet × period interaction; [m] effect of covariate; [n] $P>0.05$

Table 6. Evolution of body weight (BW) and body condition score (BCS) in Saanen goats fed high-starch (HS) and low-starch (LS) diets in mid-lactation.

	Diet	Prelimin. Period	Period				Mean	SEM[e]	*P* level			
			1[a]	2[b]	3[c]	4[d]			D[f]	P[g]	D×P[h]	π[i]
BW (kg)(kg)	HS	60.31	58.51	59.62	60.80	61.16	60.02	0.38	N.S.[l]	0.0023	N.S.	<0.0001
	LS	61.73	59.72	59.56	61.31	59.87	60.12					
BCS (0-5)	HS	2.84	2.83	2.74	2.74	2.76	2.77	0.03	N.S.	0.0530	N.S.	<0.0001
	LS	2.79	2.78	2.66	2.76	2.76	2.74					

[a] **BW**: 24 April; **BCS**: 27 April [b] **BW**: 5 May; **BCS**: 6 May; [c] **BW**: 12 May; **BCS**: 22 May; [d] **BW**: 20 May; **BCS**: 4 June; [e] standard error of the mean; [f] effect of diet; [g] effect of period; [h] diet x period interaction; [i] effect of covariate; [l] $P>0.05$

Table 7. Evolution of body weight (BW) and body condition score (BCS) in Sarda sheep fed high-starch (HS) and low-starch (LS) diets in mid-lactation.

	Diet	Prelimin. Period	Period				Mean	SEM[e]	*P* level			
			1[a]	2[b]	3[c]	4[d]			D[f]	P[g]	D×P[h]	π[i]
BW(kg)	HS	55.83	54.38	54.23	56.66	56.87	55.53	0.39	N.S.[l]	<0.0001	N.S.	<0.0001
	LS	54.76	54.92	54.11	56.54	57.04	55.65					
BCS (0-5)	HS	3.39	3.22	3.56	3.57	3.48	3.46	0.05	0.0131	0.0002	N.S.	<0.0001
	LS	3.33	3.20	3.42	3.36	3.34	3.33					

[a] **BW**: 24 April; **BCS**: 27 April; [b] **BW**: 5 May; **BCS**: 6 May; [c] **BW**: 12 May; **BCS**: 22 May; [d] **BW**: 20 May; **BCS**: 4 June; [e] standard error of the mean; [f] effect of diet; [g] effect of period; [h] diet x period interaction; [i] effect of covariate; [l] $P>0.05$

8. FIGURES

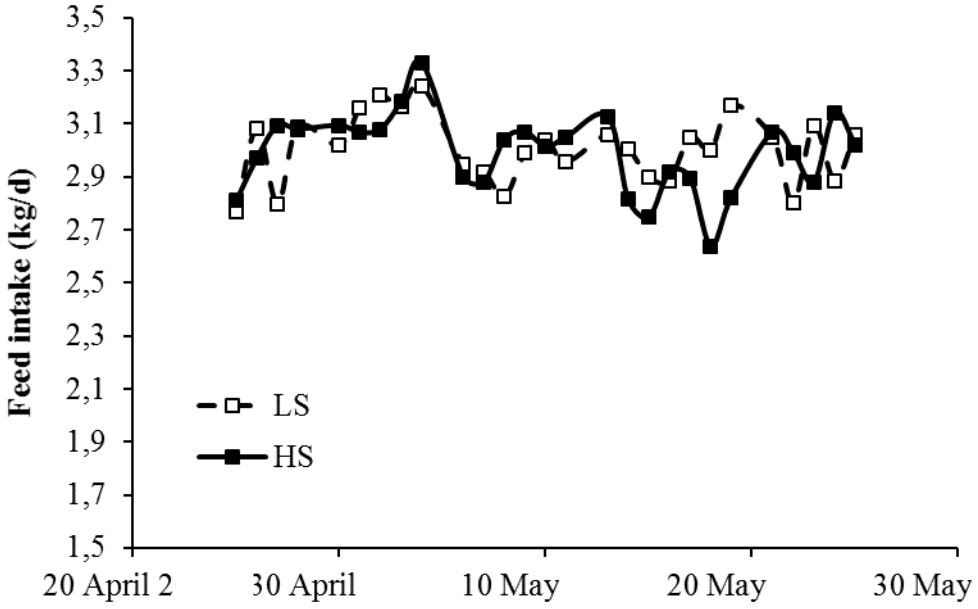

Figure 1. Evolution of feed intake (kg/d) in mid-lactating goats fed high-starch (HS) and low-starch (LS) diets during the experimental period.

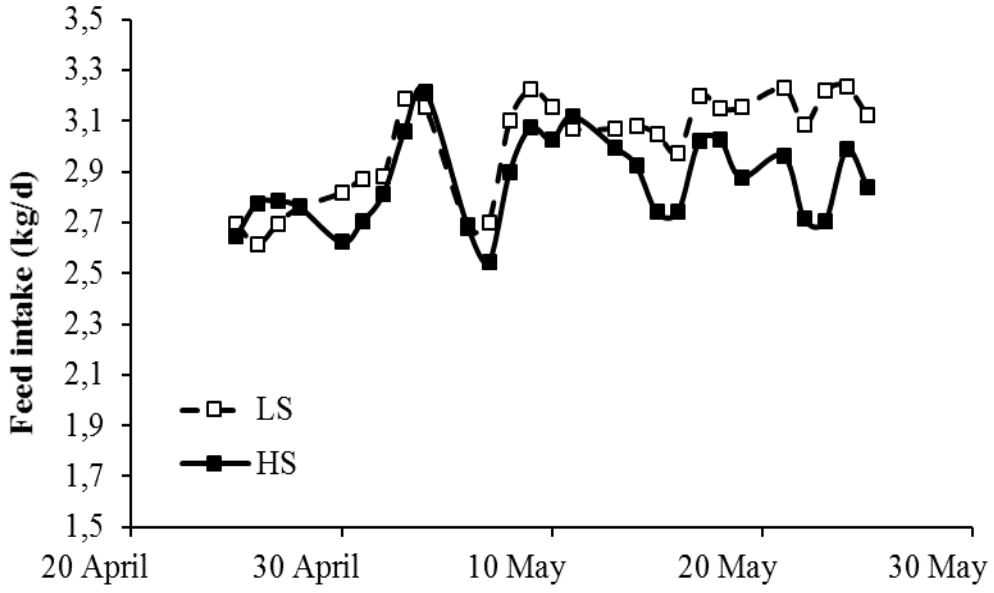

Figure 2. Evolution of feed intake (kg/d) in mid-lactating ewes fed high-starch (HS) and low-starch (LS) diets during the experimental period.

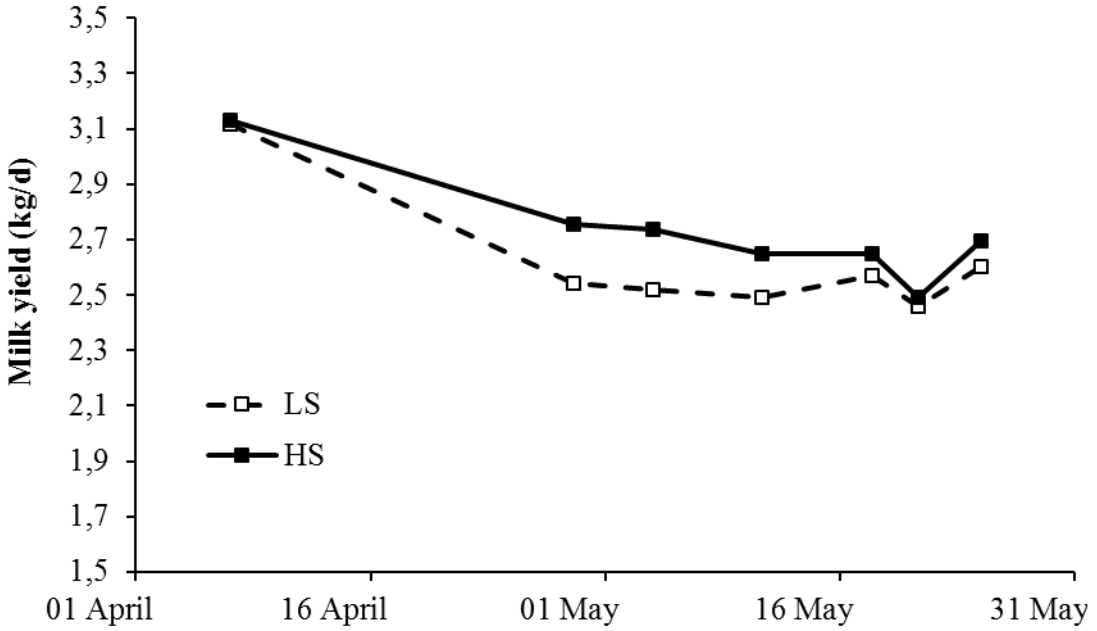

Figure 3. Evolution of milk yield (kg/d) in mid-lactating goats fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

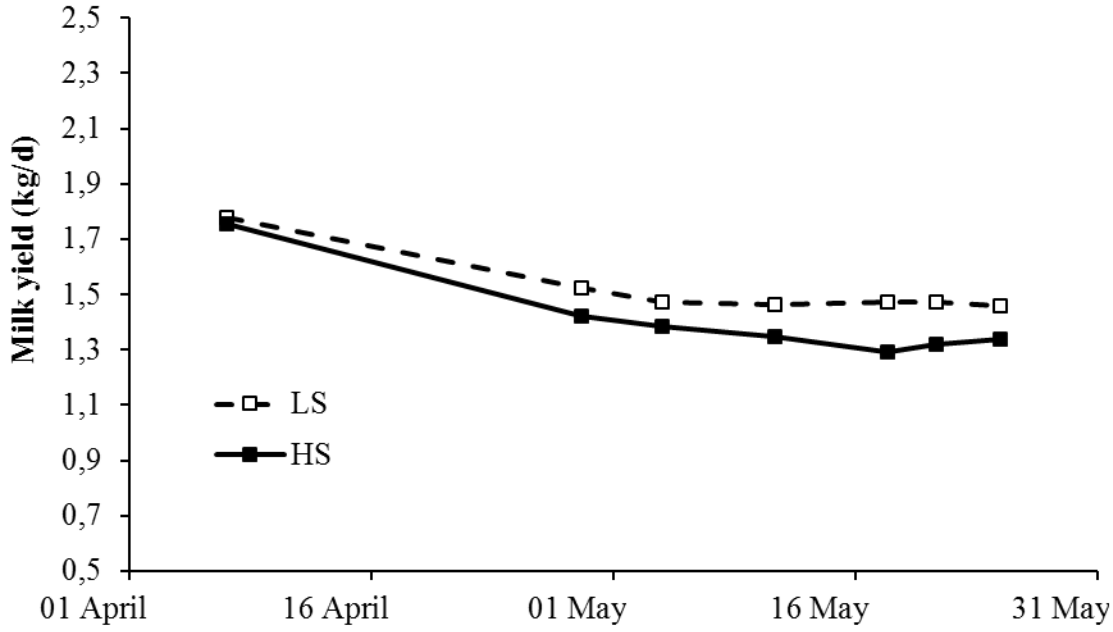

Figure 4. Evolution of milk yield (kg/d) in mid-lactating sheep fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

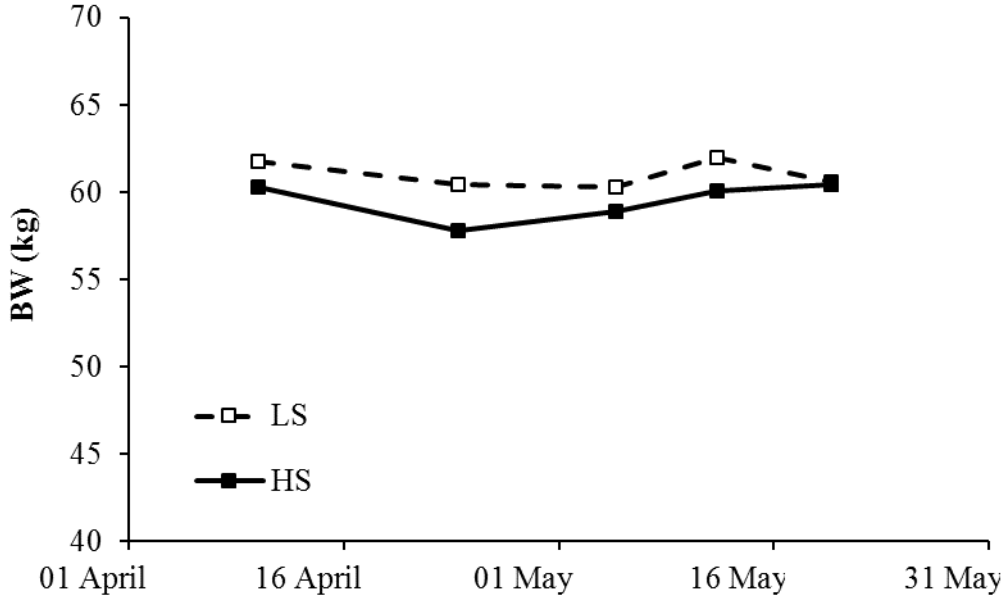

Figure 5. Evolution of body weight (BW; kg) in goats fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

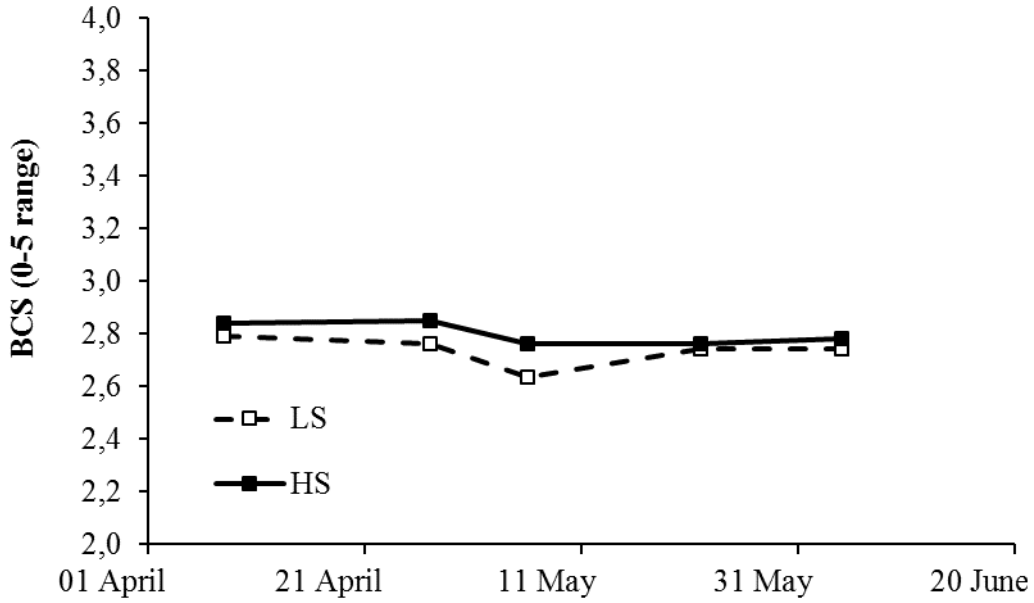

Figure 6. Evolution of body condition score (BCS; 0-5 range) in goats fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

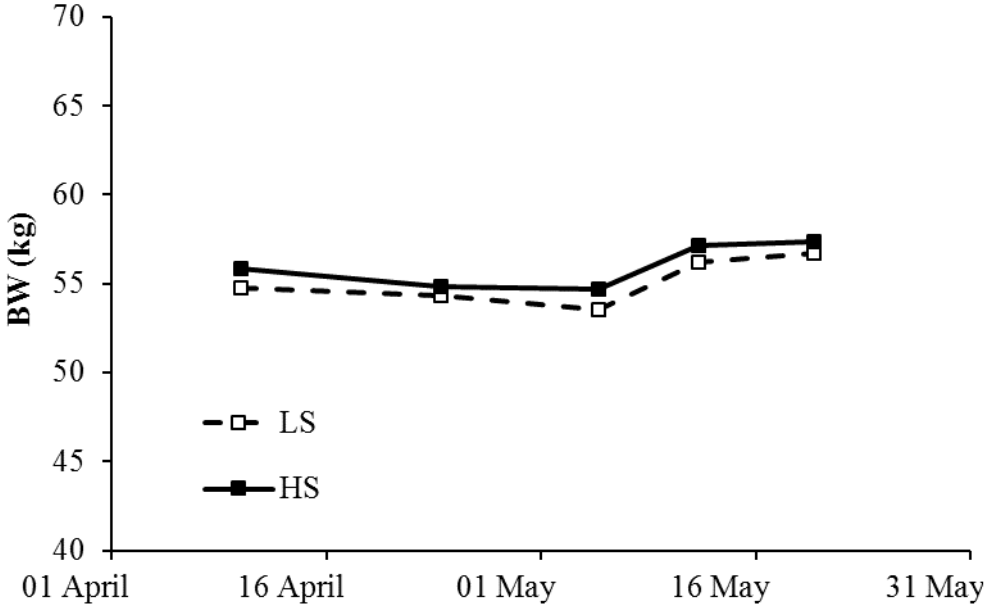

Figure 7. Evolution of body weight (BW; kg) in sheep fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

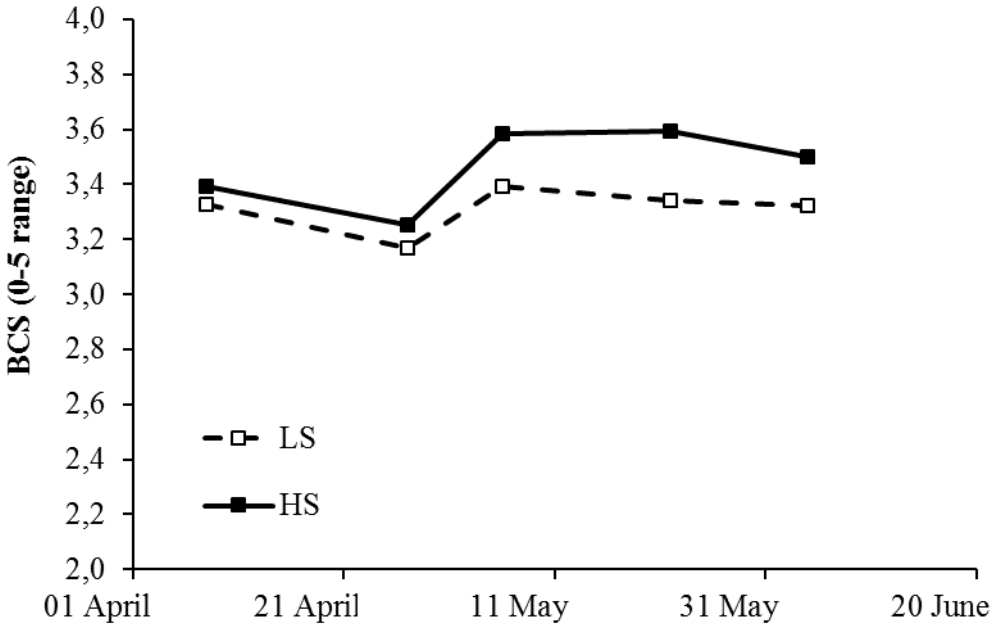

Figure 8. Evolution of body condition score (BCS; 0-5 range) in sheep fed high-starch (HS) and low-starch (LS) diets from the preliminary to the end of the experimental period.

CHAPTER 3

Metabolic and hormonal control of nutrients utilization and partitioning from early to mid-lactation in ewes and goats

ABSTRACT

The evolution of metabolic and hormonal status during the lactation and their relationships with the carbohydrates of the diet (starch or highly digestible fiber) were compared in 20 mature Sarda dairy sheep and in 20 mature Saanen dairy goats from 15 ± 5 days in milk (DIM; mean ± standard deviation) to 134 ± 5 DIM. In early lactation, each species was allocated to one dietary treatment: high-starch diet (**HS**: 20.4% starch, 35.5% NDF, 16.2% CP, on DM basis), whereas from 92 ± 11 DIM each species was allocated to two dietary treatments: HS (20.0% starch, 36.7% NDF, 15.5% CP, on DM basis) and low-starch (**LS**: 7.8% starch, 48.8% NDF, 15.6% CP, on DM basis) diets.
Once a month blood samples were collected in the morning, in order to analyze plasma glucose, non esterified fatty acids (NEFA), urea, growth hormone (GH), insulin, insulin-like growth factor 1 (IGF-I) and leptin. In addition, at the end of the trail a sequential after meal blood sampling was carried out. Data were analyzed by using the PROC MIXED procedure of SAS with repeated measurements, testing the effect of species, diet, period and their interactions (SAS Version 9.0).
From early to mid-lactation, plasma glucose concentration was higher in sheep than in goats (54.57 vs. 48.35 mg/dl ± 1.18 (mean $\pm$ SEM); $P<0.0001$). Plasma NEFA concentration was higher in goats than in ewes (0.31 vs. 0.25 mmol/L ± 0.03; $P=0.036$). Plasma urea and IGF-I concentration did not vary between species but were numerically greater in ewes than in goats (43.25 vs. 40.79 mg/dl ± 1.34; 108.77 vs. 94.24 ng/ml ± 11.64). Goats had higher plasma GH and leptin concentration and lower plasma insulin content than sheep (4.47 vs. 2.28 ng/ml ± 0.57; $P<0.001$; 26.26 vs. 11.39 ng/ml ± 2.12; $P<0.0001$; 0.11 vs. 0.26 μg/L ± 0.02; $P<0.0001$).
In mid-lactation, the hormonal and metabolic status was not affected by the diets (HS and LS) in both species, except for NEFA concentration, higher in the HS than in the LS ewes (0.15 vs. 0.09 mmol/L ± 0.02; $P=0.013$).
In conclusion, this experiment allowed to: *i)* compare the metabolic and hormonal status during the lactation of ewes and goats fed the same diet; *ii)* highlight sheep and goats have a different metabolic and hormonal profile during all lactation, with the ewes having a more insulinemic profile than goats and being more prone to accumulate body reserves; *iii)* observe that in mid-lactation the hormonal status did not vary in relationship with the type of carbohydrate (starch or digestible fiber).

Key words: nutrient partitioning, hormonal status, starch, fiber, sheep, goats

1. INTRODUCTION

Sheep and goats are both small ruminants but differ to a certain extent in their feeding behavior and selectivity, for their milk production, and possibly for their nutrients utilization and metabolism. Hofmann (1989) classified goats as intermediate feeders and sheep as grazing, differing in their feeding preferences and selectivity, being goats more prone to browse and more selective, while sheep are more devoted to grazing; Van Soest (1994) considered both species as intermediate feeders. Other differences regard their milk production. Indeed, sheep in general are less productive, with shorter lactations and produce milk with much higher fat and protein concentrations compared to goats.

Regarding the utilization of nutrients, various reports suggest that the two species respond differently to dietary carbohydrates, especially in regard to dietary sugars and starch, on one side, and fiber, on the other side. The effect of dietary carbohydrates seems to be similar in both species in early lactation, when high-starch diets have a positive effect on milk production both in dairy sheep (Cannas et al., 2002; Bovera et al., 2004) goats and dairy cattle (Chagas et al., 2009). In mid-late lactation dairy goats still respond positively to high-starch diets (Rapetti et al., 2005; Cannas et al., 2007; Ibáñez et al., 2015). In contrast, with high-starch diets dairy sheep tend to favor body reserves accumulation in respect to milk production, while when diets rich in digestible fiber (*i.e.* rich in immature forages, beet pulps, or soybean hulls) are used, milk production persistency is favored in respect to body reserve accumulation (Cannas et al., 2002; Cannas et al., 2004; Bovera et al., 2004; Zenou and Miron, 2005). These patterns were confirmed by the production results of the experiment described in Chapter 2 of this Dissertation. In particular, during mid-lactation, dairy goats had higher milk yield by using high-starch diets while dairy sheep had higher fat-corrected milk production when they used the diet with low-starch and high digestible fiber content.

These differences might be due to species differences in feeding behavior, with a better ability of goats to eat starch-rich diets in small meals compared to sheep, as suggested by Abijaoudé et al. (2000), which would be less stimulatory of insulin hikes. Another explanation could be a species difference in acetate and glucose requirements for milk synthesis, because of the different fat to lactose ratio in the milk of the two species and

the higher milk production of goats, with subsequent high lactose requirement, compared to ewes (Cannas et al., 2002).

It is also possible that the two species differ in the concentration of the hormones that controls energy partitioning or in the responsiveness of tissues involved in energy partitioning and blood glucose utilization in mid-late lactation, as a result of native differences between the two species or due to the effect of genetic selection for milk production, more intense in goats than sheep. It is well known that the physiological stage and the hormonal status during the lactation affects the energy partitioning towards fetus, mammary gland, or body reserves (Bauman and Currie, 1980; Peel and Bauman, 1987; Sasaki, 2002). Indeed, in early lactation, when the requirements of animals are high and the energy balance is negative, the nutrients are usually directed to the mammary gland, whereas later on, when the energy of diet exceeds the requirements of the animals, nutrients are stored as body fat (Bauman and Currie, 1980; Svennersten-Sjaunja and Olsson, 2005). These differences could be associated with variations in the growth hormone (**GH**) : insulin ratio, that drives nutrient partitioning toward milk production or body reserves.

Unfortunately, limited information is available on the evolution of the hormones controlling energy partitioning during the lactation of goats and sheep and no studies have compared the evolution of the hormonal status of lactating goats and ewes fed the same diet. In addition, to the best of our knowledge, possible interactions between the hormonal control of energy partition and the type of carbohydrates used in the diets have not been explored.

Thus, the aim of this work was to: *i)* study the evolution of the metabolic and hormonal status that drives nutrients utilization and partitioning during the lactation in goats and ewes; *ii)* to understand if the different response observed in sheep and goats in the use of carbohydrates (*i.e.* starch or fiber) during mid-lactation (Chapter 2) can be linked to differences in their hormonal status.

2. MATERIAL AND METHODS

The experiment was conducted at the experimental farm of AGRIS (Olmedo, Sardinia, Italy), Department of Research on Animal Production.

2.1 Experimental procedure: animals and diets

Twenty mature Sarda dairy sheep and 20 mature Saanen dairy goats were controlled from early (15± 5 days in milk (DIM; mean ± standard deviation) to mid-lactation (134 ± 5 DIM). These animals were randomly selected from a larger group (30 ewes and 26 goats), homogenous for lambing date, age (6-7 years) and milk yield that was monitored since parturition and then used in the mid-lactation feeding experiment described in Chapter 2.

The ewes and the goats were kept inside of a closed barn, in 4 large pens (2 per species, 68.4 m^2/pen), each on with an access to an external paddock (54 m^2/each). Each pen had a water through with fresh and clean water, which allowed adequate drinking space for all animals.

Before parturition all the animals were dewormed. Due to the persistency of internal parasites, a second deworming treatment was applied at 69 ± 5 DIM, with the single dosage of 15 ml per each goat and 10 ml per each sheep of albendazole (Valbazen, Pfizer Italia, Rome).

The study was divided in two periods, early and mid-lactation.

Early lactation

In early lactation, all the animals were fed a high-starch diet (**HS**; Table 1) containing, on a fed basis, 32.0% of chopped dehydrated alfalfa, 3.0% of mature ryegrass hay, and 65.0% of high-starch pellet with 30.0% starch and 27.4% NDF (all values expressed on a DM basis; Table 2). In addition, whole corn grains were supplied during the two daily milkings (200 g/d as fed, in total). In complex the diet had 35.4% NDF, 35.5% NFC, 20.4% starch and 16.2% CP; on DM basis; Table 1).

The ewes and the goats were kept with their lambs and kids until they were weaned (around 42 DIM) and then they were milked twice per day in a milking parlor.

Mid-lactation

From 92 ± 11 DIM, the animals of each species were divided in two subgroups of 10 animals each and allocated to a HS or low-starch (**LS**) diet, following the same experimental design and feeding treatment of the trial described in the Chapter 2 and of which they were part of. Within species the subgroups were balanced to have the same average body condition score (BCS) and milk production.

All the animals were fed a diet (Table 1) containing, on a fed basis, 29.0% of chopped dehydrated alfalfa, 4.0% of mature ryegrass hay, and 67.0% of the experimental pellet, which differed depending on the group as follows: i) for the HS group a high-starch pellet, with 28.1% starch and 30.7% NDF, and ii) for the LS group a low-starch pellet, composed with 10.0% of starch and 48.8% of NDF (all values expressed on a DM basis; Table 2). The pellets differed mainly because most of the corn meal and all the barley meal of high-starch pellet was replaced with soybean hulls, a high source of highly-digestible fiber, in the low-starch pellet. In addition, whole corn grains were supplied during the two daily milking (100 g/d as fed, in total).

The two diets were iso-proteic (with 15.5 and 15.6% of crude protein (**CP**) for HS and LS, respectively), whereas carbohydrates concentration differed between the two groups: 36.7% NDF, 35.4% NFC and 20.0% starch for the HS diet; and 48.8% NDF, 23.0% NFC, 7.8% starch for the LS diet, on DM basis; Table 1).

The diets were group fed *ad libitum* and were supplied twice daily (morning and afternoon) just after the two daily machine milkings, which occurred at 7:00 and 15:00.

The pellet and the alfalfa were mixed and supplied together in a large manger, which allowed free access to all animals, while the hay was supplied, at the same time, separately in another manger. Each day the orts were quantified to guarantee at least 10% of refusal.

2.2 Measurements and sampling

Once a month, before to the morning meal and after milking, blood samples were collected from the jugular vein in vacuum tubes with anticoagulants, which were lithium heparin (Venosafe VF-109SHL Lithium Heparin, Terumo Europe NV, Leuven, Belgium; 9 ml) for the subsequent determination of hormones, EDTAK3 (Venosafe VF-

053STK Terumo Europe NV, Leuven, Belgium; 3 ml) for the determination of hormones, non-esterified fatty acid (**NEFA**) and urea and with glucose-NaF/NH (Vacucheck, Nuova Aptaca s.r.l, Italy; 4 ml) for the determination of glucose. Blood samples were immediately centrifuged at 3500 rpm for 10 minutes at 4°C, to separate plasma or supernatant, that was collected and stored at -20°C until the samples were assayed. At the end of the trial, all animals were subjected to a sequential sampling of the blood during the digestibility trials carried out at the end of the lactation described in Chapter 4. In the morning, all animals were fed with half of the daily ration dose. The animals were then subjected to a sequential blood sampling carried out at 30, 60, 120, 180 and 240 minutes after the supply of the diet. The diet was continuously available for the whole sampling time. The sampling procedures followed were the same described for the monthly blood samplings.

2.3 Chemical analyses

Blood samples were analyzed for glucose, NEFA, urea, GH, insulin, leptin and insulin-like growth factor I (**IGF-I**).

Glucose, NEFA and urea were analyzed in the laboratory of Biochemistry (directed by Prof. G. C. Bomboi) of the Department of Veterinary Science, University of Sassari, Italy, and in the Laboratory of Biochemstry (directed by dr. P. Nicolussi) of the Istituto Zooprofilattico e Sperimentale di Sassari by enzymatic colorimetric assays in both species. Glucose content was analyzed by using the glucose oxidase-peroxidase (GOD-POD) method (Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China). NEFA concentration was determined by using the acyl-CoA synthetase (ACS)- acyl-CoA oxidase (ACOD) method (Wako Chemicals GmbH, Neuss, Germany). Urea content was analyzed by using urease-glutamate dehydrogenase (Urease-GLDH) in a UV method (Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China).

Leptin was analyzed through a solid phase two-site enzyme immunoassay, based on the direct sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the leptin molecule by using a goat leptin ELISA kit (Blugene Biotech, Shanghai, China) in goats and a ovine leptin ELISA kit (BluGene Biotech, Shanghai, China) in ewes. Insulin was analyzed through a solid phase two-site

enzyme immunoassay based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule by using Ovine insulin ELISA kit (Mercodia AB, Uppsala, Sweeden) for both species.

Plasma concentrations of GH and IGF-I were evaluated by radio-immuno assay (**RIA**) technique in the laboratory of Veterinary Physiology, under the direction of Prof. Alberto Prandi and the collaboration of Dr. Antonella Comin of the Dipartimento di Scienze Agroalimentari, Ambientali e Animali, University of Udine, Italy. The concentration of IGF-I in plasma samples was analyzed by RIA after an acid / ethanol extraction to release IGFs from binding proteins. The RIA was performed according to manufacturer's instructions. IGF-I was determined using an antibody distributed by Novozymes Biopharma (Thebarton, Australia). Recombinant human IGF-I (Novozymes Biopharma; Thebarton, Australia) was used as the radioligand and unlabelled ligand. The tracer was prepared with Na 125I by the iodogen method (Salacinski et al., 1981). The minimum detectable dose of IGF-I was 2.7 pg ⁄ tube. Intra- and inter-assay coefficients of variation were 4.4% and 9.1%, respectively.

Circulating bovine growth hormone was measured by a heterologous double antibody RIA, using a purified oGH preparation (LER 1774) both as the standard and tracer. The tracer was prepared following the methods described by Salacinski et al. (1981) and 10 000 cpm of the 125I-oGH solution (specific activity: 7.7 uCi/ug) obtained were added to each assay tube. The antiserum was raised in the rabbit against oGH (LER 1774) and used at the final dilution of 1/7,000. The antiserum showed a cross-reactivity of 0.1% with bovine prolactin and less than 0.01% with other pituitary hormones. Separation of free hormone from hormone–antibody complexes was achieved using an anti-rabbit gammaglobulin serum raised in the goat at the final dilution of 1/500. The sensibility of the analyses, in terms of the interpolated dose as a response to zero concentration, minus the statistical error (Programme Riastar, Canberra-Packard), was 0.28 ng/ml.

The precision of the method, within assay and between assays, evaluated with repeated doses of a sample of follicular fluid, was expressed by the coefficient of variation and resulted as 6.5% and 11.8%, respectively.

2.4 Statistical analysis

Data on metabolic and hormonal parameters were analyzed by the PROC MIXED procedure of SAS (Version 9.0, SAS Institute Inc., Cary, NC) with repeated measurements. In particular, a mixed model was used to test the differences between the species as reported in the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma + \varepsilon_{ij}$$

where: Y_{ij} is the dependent variable (i=sheep, goats; j=1-7), μ is the general mean, α_i is the effect of species (i=sheep, goats), β_j is the effect of period (j=1-7; from January 21 to May 20, 2015), $\alpha\beta_{ij}$ is the species x period interaction (i= sheep, goats; j=1-7), γ is the random effect of animal and ε_{ij} is the residual error.

Data were expressed as mean ± SEM. Means were separated using Tukey's test. The accepted level of significance was $P<0.05$.

The same model was used to test: i) the differences between the two diets, modifying α_i and $\beta_{j:}$ α_i was the effect of diet (i=HS, LS) whereas β_j was the effect of period (j=6 to 7; from May 12 to May 20, 2015 for the measurements during the trial and j = 30 to 240 min after the meal for the sequential blood samplings); ii) the differences between the two species after the change of the diet, modifying α_i and $\beta_{j:}$ α_i was the effect of species (i= sheep, goats) whereas β_j was the effect of period (j=6 to 7; from May 12 to May 20, 2015)

3. RESULTS

3.1 Evolution of milk production and body reserves

Milk production data were collected starting at an average of 48 DIM, since both the ewes and the goats were kept with their lambs and kids until they were weaned (around 42 DIM).

In goats, milk production peaked (4.5 kg/d) at 64 DIM, to slowly decrease for the rest of the lactation (Figure 1). After the application of the differentiated feeding treatments (91 DIM), milk production was higher in the HS than in the LS goats (Chapter 2). In sheep, milk production started at 2.66 kg/d and then decreased regularly during the whole lactation (Figure 1). After the application of the differentiated feeding treatment, milk production did not differ but both milk fat concentration and the 6.5% fat-corrected milk yield (**FCM**) were significantly higher in the LS than in the HS sheep (Chapter 2).
Milk fat content and milk protein content (data not reported) were much higher in sheep than in goats. Milk fat content of sheep markedly increased over the lactation from 4.4% to 6.6% at the end of the trial (Figure 2). In goats, there were little variations during the lactation and actually milk fat content was higher at the first sampling (3.7%) than in the last one (3.3%) (Figure 2). Milk protein content varied little in both species and ranged from 4.7% (first sampling) to 5.1% (last sampling) in ewes and from 3.07% (first sampling) to 3.13% (last sampling) in goats.
Body weight (**BW**) decreased until the end of the second month of lactation, then it slowly increased in both species, to reach the highest values at the end of the trial (Figure 3). The decrease in early lactation was much stronger in the goats compared to the ewes.
The BCS was lowest at parturition in both species then it very slowly increased until DIM 78 (Figure 4). After this day, it markedly increased in the ewes, to reach its maximum value at the end of the trial. In contrast, in goats the increase after DIM 78 was modest and the values were almost constant from DIM 85.

3.2 Evolution of metabolites and hormones in goats and ewes during the whole lactation

Effect of species

Plasma glucose concentration was higher in ewes than in goats (54.57 vs. 48.35 mg/dl ± 1.18 (mean ± SEM); P<0.0001) (Table 3 and Figure 5). Plasma NEFA concentration was higher in goats than in ewes (0.31 vs. 0.25 mmol/L ± 0.03; P=0.036) (Table 4 and Figure 6). Plasma urea (Table 5 and Figure 7) was not affected by species but was numerically slightly greater in ewes than goats (43.25 vs. 40.79 mg/dl ± 1.34; for ewes and goats; respectively). Goats had higher plasma GH (4.47 vs. 2.28 ng/ml; P=0.0004; Table 6 and Figure 8) and lower insulin concentration (0.11 vs. 0.26 µg/L ± 0.02; P<0.0001. Table 7 and Figure 9) than sheep. The IGF-I concentrations (Table 8 and Figure 10) were not affected by species but were numerically greater in ewes than goats (108.77 vs. 94.24 ng/ml ± 11.64; for ewes and goats; respectively). Leptin was higher in goats than sheep (26.26 vs. 11.39 ng/ml; P<0.0001. Table 9 and Figure 11).

Effect of period

The effect of period was statistically significant for plasma glucose (Table 3), NEFA (Table 4), urea (Table 5), GH (Table 6), and insulin (Table 7), IGF-I (Table 8), and leptin (Table 9)

Effect of species x period interaction

The species x period interaction was statistically significant for plasma glucose at 126 and 134 ± 5 DIM (P=0.003 and 0.045; respectively; Table 3), NEFA (P=0.005; Table 4), urea (P<0.0001) at 15 and 49 ± 5 DIM (P=0.036 and <0.0001; respectively; Table 5), GH (P=0.012) at 49 ± 5 DIM (P=0.02; Table 6), insulin (P=0.008) at 15 (P=0.009), 49 (P=0.036), 78 (P=0.021), 126 (P=0.01) and 134 ± 5 DIM (P=0.004) (Table 7), IGF-I (P=0.0003) at 15 ± 5 DIM (P=0.04; Table 8), and leptin (P=0.0011) from 15 to 134 ± 5 DIM (Table 9).

3.3 Effects of HS and LS diets and animal species in mid-lactation on the metabolic and hormonal status

Effects of diet, period and their interactions

In Saanen goats, blood glucose was not statistically different between the two diets but it was numerically greater in the HS than in the LS goats (49.16 vs. 47.38 mg/dl ± 2.39; Table 10). NEFA was not statistically different between the two diets but was numerically lower in the HS than in the LS goats (0.15 vs. 0.19 mmol/L ± 0.04; Table 10). Urea did not vary between the two diets but was numerically greater in the HS than in the LS goats (46.02 vs. 43.32 mgl/dl ± 2.70; Table 10). Similarly, GH was not statistically different between the two diets but was numerically lower in the HS than in the LS goats (2.05 vs. 3.18 ng/ml ± 1.09; Table 10). Insulin was not statistically different between the two diets, but was numerically higher in the HS than in the LS goats (0.16 vs. 0.12 μg/L ± 0.03; Table 10). IGF-I (Table 10) and leptin concentration did not differ between the two diets (Table 10).

In Sarda ewes, blood glucose was not statistically different between the two diets (Table 10). NEFA was lower in the LS than in the HS ewes (0.09 vs. 0.15 mmol/L ± 0.02; P=0.013; Table 10). Urea did not vary between the two diets (Table 10). GH as well was not statistically different between the two diets (Table 10). Insulin was numerically lower in the HS group than in the LS group (0.34 vs. 0.42 μg/L ± 0.08; Table 10). IGF-I content did not vary with diet but was numerically lower in the LS than in the HS ewes (109.76 vs. 129.96 ng/ml ± 15.03; Table 10). Leptin concentration did not differ between the two groups (11.66 vs. 12.28 ng/ml ± 1.86 for HS and LS, respectively; Table 10).

In goats, the effect of period was statistically significant for GH (P=0.022; Table 10) and leptin (P=0.046; Table 10), whereas it was not significant for glucose, NEFA, urea, IGF-I and insulin (Table 10). In sheep, the effect of period was never significant.

In both species, the diet x period interaction was not significant for any of the blood parameters considered (Table 10).

Effect of species, period and their interactions

After the application of the experimental diets, plasma glucose concentration continued to be higher in ewes than in goats (56.0 vs. 48.3 mg/dl ± 1.7 (mean ± SEM); P<0.0001) (Table 11). Plasma NEFA concentration was not affected by species but was numerically greater in goats than ewes (0.16 vs. 0.12 mmol/L ± 0.02; P=0.098) (Table 11). Plasma urea and IGF-I concentrations (Table 11) were not affected by species but IGF-I was numerically greater in ewes than goats (119.7 vs. 100.8 ng/ml ± 13.4; for ewes and goats; respectively). Goats had higher plasma GH (2.62 vs. 1.37 ng/ml ± 0.58; P=0.038; Table 11) and leptin (24.72 vs. 11.97 ng/ml ± 2.13; P<0.0001; Table 11) concentrations and lower insulin concentration (0.14 vs. 0.38 µg/L ± 0.05; P<0.0001. Table 11) than sheep.

The effect of period was statistically significant only for GH (P=0.01; Table 11).

The species x period interaction was statistically significant only for plasma GH (P=0.041; Table 11).

After meal sequential sampling at the end of the lactation

The diet effect was not significant for any of the metabolites and hormones studied in the sequential sampling. For this reason, only the comparison between species was considered.

The effect of animal species was always significant except for NEFA (glucose: P<0.0001; NEFA: P>0.05; GH: P<0.02; insulin: P<0.04; IGF-I: P<0.002; leptin: P<0.001; Figure 12). The effect of sampling time was significant only for leptin (P<0.012) and close to significance for GH (P< 0.06) (Figure 12).

The species x diet x sampling time was significant for glucose (P<0.0001) and blood urea (P<005) (Figure 12).

4. DISCUSSION

4.1 Performances of the animals

Evolution of milk production and composition during the lactation

The pattern of milk production was in line with the lactations curves for ewes and goats described in the literature for these species. Since the lambs and the kids were kept with their mothers in the first moth and half of lactation, it was not possible to measure milk production in that period and thus to observe if just after parturition there was an ascending phase of the lactation curve.

Milk production was high in both species during the whole experiment. It is well known that dairy goats of the Saanen breed have very high milk production and persistency. The milk production values for the Sarda ewes used in this experiment was higher than that usually observed for this breed. Indeed, the ewes used had all a high genetic merit, being part of the selection flock of the Sarda breed.

Milk fat concentration in early lactation was in line with the normal values for the Saanen goats but it was much lower than the expected values in the case of the Sarda ewes. Likely this was a result of the combined effect of very high milk yield and of the high-starch content of the diets used in early lactation (Table 1).

After the application of the differentiated feeding treatment, milk production did not differ but both milk fat concentration and the 6.5% FCM were significantly higher in the LS than in the HS sheep (Chapter 2).

Evolution of body weight and body condition score during the lactation

Body weight and BCS evolved differently in the two species.

In sheep, it BW decreased slowly in the first two months of lactation, then it markedly increased until the end of the experiment. BCS, instead, increased during the whole experiment, with a marked increase after the second month of lactation.

In goats, there was a dramatic decrease in BW in the first two months of lactation, then it increased until the end of the experiment. BCS did not show significant variations until 80 DIM and there was only a slightly increased thereafter.

This data showed a lack of concordance between BW and BCS in early lactation. Probably BW decreases not because of changes in body fat reserves but as an effect of reduction in weight of the uterus and possibly a loss of body proteins and the associated body water. In contrast, both BW and BCS variations suggested an accumulation of body reserves after the second month of lactation.

Effect of the feeding treatments applied in mid-lactation

The applications of the differentiated diets after DIM 91 caused important variations in milk yield and composition. The goats produced more milk with the HS diet than the LS diet. In the ewes milk production did not differ but both milk fat concentration and the 6.5% FCM were significantly higher with the LS diet (Chapter 2).

The BCS did not differ between HS and LS fed goats, while in the case of ewes it was significantly higher for those fed the HS diet.

Overall, these differences indicate a different response to the same nutrients between the two species. Possible metabolic and hormonal mechanism causing this differences will be discussed in next paragraph.

4.2 Evolution of metabolites and hormones during the whole lactation of ewes and goats

The experiment evidenced important differences in the metabolic and hormonal profile and its evolution over the lactation between sheep and goats.

Glucose

In this study glucose concentration was significantly higher in the ewes than in the goats (Table 3 and Figure 5). Even though the effect of period was significant, the variations in glucose concentration during the lactation were fairly limited. In both species, the value was highest in early lactation, especially at DIM 21 and 49, then decreased until DIM 94, to increase again, especially in sheep, at the end of the experiment (DIM 126 and 134).

The differences in terms of blood glucose content observed between ewes and goats are independently by the differences in milk production, much higher in goats than ewes. In

fact, some studies suggested that blood glucose increases with the production level of animals (Karapehlivan et al., 2007) but only comparing animals belonging to the same species. Therefore, the greater level of blood glucose content observed in the ewes than in the goats does not mean that sheep are more productive than goats but that glucose concentration and is regulation differ between goat and ewes, independently by their production level. Probably, the lower blood glucose concentration observed in the goats than in the ewes can be attributed to its utilization by the mammary gland, that can be greater in goats than ewes. Indeed, due to their much high milk production, the goats in the experiment had a much higher daily lactose output than the ewes. Blood glucose content has an important function on milk synthesis (Bell and Bauman, 1997) because is the most important substrate used by the mammary gland (Oddy et al., 1985) for lactose synthesis (Van Soest, 1994). For the same motivation, mammary gland is identified as dependent glucose-organ (Zhao and Keating, 2007). The greater the amount of glucose absorbed, the greater the amount of lactose synthesized, the greater amount of milk produced (Cannas et al., 2002; Pulina et al., 2005). Previous studies observed that blood glucose concentration was lower in pregnant than non-pregnant goats probably due to its utilization by the growing fetus, as suggested by the authors (Khan and Ludri, 2002). The amount of glucose up taken by mammary gland, as reported by De Koster and Opsomer (2013), is the results of intensive genetic selection. In fact, being glucose an energy sources, milk production causes an important drain of glucose (Veerkamp et al., 2003). So, it is possible that high yielding animals subjected to intense genetic selection, as can be considered goats compared to sheep, have low glucose level (Veerkamp et al., 2003). However, previous studies did not observe differences in glucose concentration between high and low genetic merit cow for milk production (Barnes et al., 1985). The intense genetic selection seems to have an influence in terms of insulin resistance. In fact, dairy cows with high genetic merit for milk production seem to be more insulin resistant or, in other words, to have lower peripheral glucose concentration for a reduced insulin sensitivity of tissues (De Koster and Opsomer, 2013). In ruminant species, glucose production depends mostly on hepatic gluconeogenesis (Bell and Bauman, 1997) because, in contrast to what occurs in monogastric species, the amount of glucose absorbed in the intestine is low. Both in sheep and goats glycemia values were lower compared to non-ruminant species, which have values around 80-100 mg/dl,

as reported by Van Soest (1994) and by other authors (De Koster and Opsomer, 2013). In fact, "the decrease in blood glucose concentration seems to be an evolutionary adaptation of the adult ruminant to the perpetual necessity of carbohydrates conservation" (Van Soest, 1994). Adult ruminant maximize glucose conservation through different mechanisms, which do not occur in non-ruminant, and that inhibit the direct conversion of glucose to acetate and fatty acid in ruminant tissues (Van Soest, 1994). Probably, the lower glycemia value observed in the goats than in the ewes can be due to the necessity of the goats to conserve carbohydrates to sustain milk production, that requires large amount of lactose. For the same motivation, it is possible that sheep use more glucose for lipogenesis and body reserves accumulation compared to goats. Other studies reported that the reduction of blood glucose content is an indicator of negative energy balance and that glucose tends to increase during the lactation, having maximum levels in positive energy balance (Adewuyi et al., 2005). Indeed, glucose concentration increased ($P<0.05$) during the lactation in Tsigai sheep at 20, 40 and 60 daily in milk (3.26, 3.86, and 4.30 mmol/L at 20, 40 and 60 DIM, respectively) (Antunović et al., 2011). Basal glucose utilization was higher in lactation than dry period and in early lactation than in mid-lactation (321 ± 18 vs. 265 ± 14 mg/min; $P<0.05$) in Alpine goats (6-7 years old) subjected to euglycemic-hyperinsulinemic clamp (Debras et al., 1989). The same authors suggested that changes in glucose metabolism was linked to mammary glucose uptake (Debras et al., 1989). This trend was not observed in our experiment (Figure 5): even though glucose content changed during the lactation, with a significant effect of period ($P<0.0001$), the lowest values were observed in mid-lactation and the values in late lactation were only slight higher than in early lactation (Table 3, Figure 5). No clear hypothesis can be drawn on this regard to explain this pattern, except that as lactation progressed, environmental temperature increased and intake decreased, probably reducing the overall availability of glucose precursors.

The values of glucose observed in our experiment are in accordance (Yokus et al., 2006) or lower than the values found in the literature for ewes (El-Sherif and Assad, 2000) and are in accordance with the values of glucose observed for goats (Pambu-Gollah et al., 2000) in different experimental conditions.

NEFA

The NEFA concentration was higher in goats than in sheep, with much higher values, for both species, in early than mid-lactation (Table 4, Figure 6).

The higher NEFA content observed in goats compared to sheep confirmed the important energy requirement of this species to sustain milk production, probably in part achieved through the mobilization of body reserves. As reported by Tedesco et al. (2008), the release of NEFA from adipose tissue to blood circulation occurs to sustain energy requirements, generally when glucose level fall (Veerkamp et al., 2003). In fact, among blood metabolites, NEFA and glucose, can be considered the most important nutrients indicators of the energy balance (Khan and Ludri, 2002). In analogy to what observed for glucose, NEFA concentration tends to be high in cows with high genetic merit for milk production (Hart et al., 1978; Barnes et al., 1985; Veerkamp et al., 2003; Weber et al., 2007). Indeed, as reported by Veerkamp et al. (2003), high genetic merit animals mobilize more body tissues during their lactation. However, some studies suggested that there is not relationship between NEFA and genetic selection (Westwood et al., 2000; Roche et al., 2006). Probably sheep, compared to goats, are more inclined to body fat deposition than fat mobilization, as observed in some comparative studies between the two species (El Khidir et al., 1998; Tshabalala et al., 2003; Sen et al., 2004). There was a marked reduction of NEFA during the lactation in both species (Figure 6), as observed by the highly significant effect of period ($P<0.0001$; Table 4). This pattern was probably due to the evolution of the energy balance of the animals and was specular, as expected, to that of BCS, confirming that NEFA can be considered good indicators of energy balance (Adewuyi et al., 2005).

The values of NEFA observed in our experiment are in accordance with the values found in the literature both for ewes (Sevi et al., 2001; Chiofalo et al., 2005) and for goats (Khaled et al., 1999; Bava et al., 2001; Piccione et al., 2010) in different experimental conditions.

Urea

Blood urea did not differ between the two species. Urea synthesis is the most important mechanism to excrete ammonia and its blood concentration depends on protein catabolism. So, the absence of differences can be linked to the CP level of the diets, that

was the same for all diets. Plasma urea slightly increased during the lactation in both species (Figure 7), as indicated by the significant effect of period (P<0.0001; Table 5). This was probably associated to the decrease in milk production, and thus in the protein requirements, which caused an increase in dietary protein wastage. The effect of genetic selection and thus on milk production on blood urea is not clear, and it has been associated to low urea content (Diab and Hillers, 1996; Veerkamp et al., 2003), with no effect (Westwood et al., 2000; Veerkamp et al., 2003), or a tendency to increase (Barnes et al., 1985; Veerkamp et al., 2003).

The values for ewes are higher than those reported by Cannas et al. (1998) for ewes with eating diets with about 15% CP, which was the average for this experiment. Similarly, the values for goats are higher than those reported by Rapetti et al. (2005). Thus, for both species, it is likely that CP was in excess of the requirements.

Growth hormone

The concentration of GH was higher in goats than ewes for the whole lactation, with the exception of the first sampling day (15 DIM). The values of goats had a very high concentration at 49 DIM, not observed in the ewes, then the concentration decreased in both species.

The higher values of GH observed in goats than ewes are probably associated to the higher milk yield of this species compared to that of ewes measured in our experiment, confirming the important galactopoietic activity of this hormone (Neville et al., 2002). In fact, as suggested by several studies, GH plays a crucial role in the partitioning of nutrients toward milk production but only if milk production is sufficiently high, while the effects are limited if the animals are not much productive (Hart et al., 1978; Hart, 1983). Probably, the highest value of GH observed in goats can be due, also, to the genetic selection for milk production to which Saanen goats have been subjected. As suggested by Kazmer et al. (1986), high GH concentration is the results of the intense genetic selection. In fact, in highly yielding cows under intense genetic selection, the content of blood GH generally is high as confirmed by several authors (Barnes et al., 1985; Kazmer et al., 1986; Westwood et al., 2000; Veerkamp et al., 2003; Weber et al., 2007). However, others studies did not find differences (Diab and Hillers, 1996; Veerkamp et al., 2003). GH is a peptidic hormone discovered in 1920 by Evans and

Simpson. The mechanism behind the GH activity on milk production is not well defined. McDowell et al. (1987) excluded the direct effect of GH on the mammary gland of sheep and goats when the somatotropic hormone was injected to the animals. Several authors suggested that GH increases the availability of milk precursors, stimulating lipolysis and glucose and acetate partitioning from peripheral tissues to the mammary gland (Welt and Wilhelmi, 1950; Luft and Guillemin, 1974; Hart, 1983; Davis and Collier, 1985). As reported by Peel and Bauman (1987), when GH concentration is high, nutrients are directed to mammary gland, in opposition to what observed when it concentration is lower. According to its lipolytic effect, which increases the amount of reserves mobilized, GH could be responsible of the higher NEFA content observed in the goats than in the ewes of this experiment, as previously suggested by Aguggini et al. (1998). GH content changed during the lactation (Period effect: $P<0.0001$; Table 6, Figure 8), according to what reported in the literature. Indeed, GH levels are high in early lactation and tend to decrease with as the lactation progresses and with the associated reduction of milk production (Kazmer et al., 1986; Petitclerc et al., 2000; Cannas et al., 2002). In particular, the high values of GH in early lactation are associated to an insulin resistance status that occurs to drive nutrients toward mammary gland. In fact, high GH levels inhibit the lipogenic action of insulin (Bauman and Vernon, 1993; Bell and Bauman, 1997). With the advancement of the lactation, GH concentration decreases, milk production is reduced, in association to a status of insulin sensibility. However, in the literature there are not reports that describing the evolution of GH during the whole lactation of dairy ewes and goats. The information available is fragmented to specific stages and short periods, as reported by Cannas et al. (2002).

The values of GH observed in our experiment are in accordance with the values found in the literature for dry ewes (Matsunaga et al., 1998) and lactating ewes (Pulina et al., 2012; Cannas et al., 2013) and are in accordance (Jin et al., 2012) or lower (Singh and Ludri, 2002) than the values observed for female goats in different experimental conditions.

Insulin

Insulin concentration was significantly higher in the ewes than in the goats. This suggests that the goats present a metabolic status more inclined toward a catabolic pathway to sustain milk production.

Some studies reported that animals selected for milk production have low insulin levels (Gutierrez et al., 1999), in others no differences between high and low genetic merit animals for milk production were observed (Barnes et al., 1985; Westwood et al., 2000; Veerkamp et al., 2003). In fact, insulin is involved in the nutrients deposition (Rosi et al., 2009), through the regulation of the glycemia, removing glucose from blood circulation when its level exceeds the optimal range (Duque-Guimarães and Ozanne, 2013) and increasing cellular glucose uptake (Baumgard et al., 2016).

In our experiment, insulin level changed during the lactation ($P<0.0001$; Table 7), increasing after 78 DIM (Figure 9) in both species, even though this was most pronounced in the ewes. In the ewes, the marked insulin increase after the peak of lactation was associated to concomitant marked body reserves accumulation. A similar pattern occurred in goats, even though both the insulin increase and the body reserve increase was much milder in this species compared to the ewes.

These patterns are in agreement to data on Alpine goats (6-7 years old), where insulin concentration did not differ in early lactation compared to the dry period but increased ($P<0.05$) during mid-lactation (Debras et al., 1989) and to data on ewes of the Tsigai breed monitored at 20, 40 and 60 days of lactation, where insulin levels increased over time (Antunović et al., 2011).

Insulin seems to be inversely related with milk production level (Lomax et al., 1979). In particular, in early lactation blood insulin levels are low (Karapehlivan et al., 2007) and begin to rise with the advancement of the lactation (Lomax et al., 1979; Bell and Bauman, 1997; Sasaki, 2002; Fiore et al., 2014), increasing at the same time the insulin sensitivity of the tissues. Probably, during periods of positive energy balance, goats are less insulin sensitive compare to ewes, to sustain their usually high milk production.

The values of insulin observed in our experiment for sheep are in accordance (Pulina et al., 2012) or lower (Bichi et al., 2013) than the values observed in the literature for lactating ewes in different experimental conditions. The values of insulin observed in

our experiment for goats are lower than the values observed in the literature for lactating (Avondo et al., 2015) or non-pregnant goats (Gallego-Calvo et al., 2015) in different experimental conditions.

Insulin-like growth factor I

The IGF-I concentrations differed only numerically between the two species, with the highest values in sheep for most of the lactation, and changed during the lactation (effect of period: P=0.0005; Table 8). The values of sheep were substantially higher than those of goats in first two months of lactation, while were more similar later on.

The insulin-like growth factors, or somatomedins, are peptides produced through the control of GH. In particular, IGF-I is produced by the liver (Aguggini et al., 1998). Probably, IGF-I, in contrast to what observed for GH, is involved in fat deposition but not in milk production. In human studies, Oliveira et al. (2011) suggested that, even though IGF-I is produced under GH control, it goes in opposite direction; it seems that GH reduces insulin sensitivity whereas IGF-I increases it. In other words, IGF-I has an insulin complementary activity and follows an anabolic pathway, even though it is produced through GH control, which instead has a catabolic effect (Veerkamp et al., 2003). The association between IGF-I and insulin could be linked because of IGF-I is involved in the development of the β cells of pancreas (involved in the insulin synthesis) (Oliveira et al., 2011). So, the lower level of IGF-I found in the goats compared to the sheep was expected according to what found in the literature, where IGF-I seems to be low in high genetic merit cow (Veerkamp et al., 2003; Weber et al., 2007). In the goats of our study, IGF-I was lowest at early lactation and tended to increase during lactation (Figure 10). IGF-I, being an anabolic hormone that favors lipogenesis, probably increases during periods positive energy balance and in mid-late lactation. Thus, the high IGF-I concentration in mid-late lactation is associated to high insulin sensitivity status, that occurs in animals in positive energy balance.

The values of IGF-I observed in our experiment are in accordance with the values found in the literature both for ewes (Cannas et al., 2004; Banchero et al., 2006; Pulina et al., 2012) than for goats (Faulkner, 1999) in different experimental condition.

Leptin

The leptin concentration was much higher in goats than in sheep for the whole experimental period. This is in accordance to the evolution observed for NEFA, which were also highest in goats. In fact, leptin is a hormone that contrast lipogenesis (Maury and Brichard, 2010), in addition to other function, *i.e.* regulates satiety sense (Zhang et al., 1994; Vernon et al., 2001), decreases food intake (Gámez-Vázquez et al., 2008), increases glucose uptake (Maury and Brichard, 2010) and is correlated with high glycemic value (Di Palo et al., 2005). However, some studies suggested that leptin was correlated with body fat in sheep (Chilliard et al., 1998; Blache et al., 2000) and with BCS in Criollo goats (Gámez-Vázquez et al., 2008). In addition, it seems that differences in leptin concentration are linked to differences in the capacity to accumulate body fat; *i.e.* in ewes, leptin expression was linked more to the subcutaneous fat than the abdominal fat (Di Palo et al., 2005). Some variations occurred during the lactation (Figure 11), with the effect of period that was statistically significant (P=0.005; Table 9). Indeed, the variation were evident in goats only, which had an increase in leptin concertation after the first 3 weeks of lactation. The values decreased again at the end of the lactation. In contrast, the values of the ewes were almost constant for the whole lactation.

The literature reports that leptin is generally positively associated to the energy balance of the animals, being low in early lactation, due to losses of body fat associated to body reserve mobilization (Reist et al., 2003) or to low insulin concentration (Block et al., 2001), according to an insulin resistance status that occurs in early lactation. In early lactating cows, the low value of leptin permits to reduce the response of peripheral tissues to insulin, favoring the use of glucose by mammary gland (Bell and Bauman, 1997; Etherton and Bauman, 1998).

The values of leptin observed in our experiment are higher than the values observed in the literature for goats (Bonnet et al., 2009) in different experimental conditions. For sheep, the values observed in our experiment are lower than the values reported by Bichi et al. (2013) but higher than those reported by Cannas et al. (2004).

4.3 Effect of the utilization of HS and LS diet in mid-lactation on the evolution of metabolites and hormones

The type of the diet (HS vs. LS) did not affect the metabolites and the hormones studied both in sheep and in goats, excepts for the NEFA content, that in ewes was lower with the LS than the HS diet. Similarly, no diet effects were observed on metabolites and hormones when their short-term evolution after a meal was observed.

Glucose

The absence of differences on glucose concentration was not expected, since the HS diet should have promoted high gluconeogenesis. In addition, there were differences in milk production level within species and this should have also caused differences in glucose concentration. Indeed, according to Karapehlivan et al. (2007), glucose plasma concentration increases with the production level of the animals. In this sense the literature is not consistent. In Saanen goats fed a no-forage diet (NDF: 36.8%; NFC: 28.8%, on DM basis) or a control diet richer in NFC (NDF:33%; NFC: 40.6%, on DM basis) there were not differences in glucose concentration both in mid-lactation and late lactation (Bava et al., 2001). In contrast, glucose concentration was higher in mid lactating Sarda sheep fed a high-starch diet (36% NFC, on DM basis) compared to a low-starch diet (23% NFC, on DM basis) (Cannas et al., 2013) or in mid lactating ewes fed a high-starch diet compare to high NDF diet (Cannas et al., 2004), despite in both experiments milk production was higher in the low than in the high-starch diets. The sequential after meal pattern of glucose confirmed the lack of effects due to the diets in both species, even though in goats but not in sheep the value for HS diets were numerically higher than for LS diets (data not reported). Interestingly, Cannas et al. (2004) reported a marked after meal increase in blood glucose (from 63 to 80 mg/dl) but most of the increased occurred starting after hour after the meal, a time frame not explored in our study.

The species difference observed in early lactation was confirmed in mid-lactation and in the sequential after meal measurements, with sheep having higher values than goats. This species difference was discussed in the previous paragraph and it is probably

associated to the species differences in genetic merit for milk production, with goats having a higher drain of glucose as milk lactose than sheep.

NEFA

In goats, NEFA concentration did not differ between the two diets, whereas it was highest with HS diet in sheep, while Cannas et al. (2004) found higher values in mid lactating ewes fed a low-starch diet compared to a high-starch diet. In other previous experiments, NEFA content did not vary between high and low-starch diets both in goats and in sheep (Bava et al., 2001; Cannas et al., 2013). The higher NEFA concentration of the ewes fed the HS starch diet was not confirmed in the sequential after meal measurements carried out at the end of the experiment (Figure 12). In any case, the differences even though significant were numerically low and thus of little biological significance. Both the measurement in the experiment sampling days and the sequential after meal sampling indicated a tendency ($P<0.1$) of goats of having higher values than ewes (Table 11 and Figure 12). This is in line with the fact that goats accumulated less body reserves (lower BCS increase) than the ewes, which instead had a marked increase of BCS throughout the mid-lactation stage.

Urea

In both species blood urea did not differ between the HS and LS diets, both in the morning blood samplings and in the sequential sampling. This is in agreement with the values on milk urea reported in Chapter 2, which were also similar between diets. Even though sometimes low-starch diets have higher blood or milk urea values (e.g. Cannas et al., 2013), for the shortage of energy available to the rumen microbial population, which reduces their ammonia utilization for microbial synthesis, in our experiment probably even the LS diets provided enough energy at rumen level.

Growth hormone

Growth hormone did not differ between the two diets in both species, but it was numerically higher in the LS than the HS goats. In previous studies conducted on sheep, GH was highest with a low than a high-starch diet (Cannas et al., 2004) or did not vary between the two diets (Cannas et al., 2013), even though this latter result was difficult

to explain, as suggested by the authors. GH concentration in mid lactating goats was higher for high amylose starch (the least degradable starch at rumen level) than for normal starch diets, at equal dietary starch concentrations (2.55 vs. 1.65 ng/ml; P=0.06; Wang et al., 2016), suggesting that GH is increased by diets which provides less energy at rumen level. Hagino et al. (2005) found that increasing the dose of concentrate fed to wethers there a proportional decrease of GH concentration before and after the meals. Hatfield et al. (1999) found that GH was similar in underfed than fully fed lactating ewes before the meal, but it was higher in the underfed ewes after the meal.

Regarding species differences, GH was higher in goats than ewes both in the regular morning samplings and in the sequential after meal samplings. Interestingly, as in Cannas et al. (2004), GH decreased, except for the HS sheep, in the first hour after the meal and then increased. This pattern was particularly evident in goats, with the LS goats that increased their GH before the HS diets. At 3 hours after the meal GH concentration was twice as high as one hour after the meal. A similar pattern, *i.e*. GH decrease just after the meal and then increase, was found by Hatfield et al. (1999) in lactating ewes fed at libitum, while in the same research overfed dry ewes for many hours decreased GH after the meal. Similarly, Hagino et al. (2005), in wethers fed increasing doses of concentrates, found that after the meal GH decreased for a long time and its recovery was inversely proportional to the dose of concentrate used. It seems that the decrease of GH after meal is limited in time in animals with high requirements, such as the goats in our experiment or the lactating ewes of Hatfield et al. (1999), and it lasts for longer time in dry or low requirement animals, such those of Hagino et al. (2005), the dry ewes of Hatfield et al. (1999) and the lactating ewes in our experiment.

Insulin

Insulin did not vary between the two diets in both species (Table 10), even though the sequential after meal measurements showed (Figure 12) that sheep fed HS had a pick of insulin just after the meal. This was not observed for LS sheep and for both dietary groups of goats. The limited effects of diets on insulin concentration are difficult to explain and are somehow in contrast to what observed in the literature, where high-starch diet increased insulin concentration in Saanen goats (Magistrelli et al., 2005) and in Sarda ewes (Cannas et al., 2004). At equal dietary starch concentrations, insulin

concentration in mid lactating goats was higher for high amylose starch than for normal starch diets, (15.21 vs. 11.70 μ IU/ml; P=0.03) (Wang et al., 2016), possibly because a higher proportion of amylose starch escaped the rumen and was then digested in the small intestine. Thus, insulin can be modulated non only by ruminal propionate production but also by the starch that escapes.

Insulin was significantly and markedly higher in sheep than goats, both in the regular morning samplings and in the sequential after meal samplings. In the latter, while just 30 after the meal the differences were limited, at one hour and until 3 hours after the meal the sheep values were higher than the goat values.

Insulin-like growth factor I

Insulin-like growth factor I did not differ between the two diets in both species, even though in the ewes it was 20 ng/ml (P was > 0.05) higher for HS than LS diets. A similar trend was observed in the after meal sequential analysis (Figure 12). On this regard the literature is contrasting. Magistrelli et al. (2005) observed a higher IGF-I concentration in Saanen goats fed a high-starch diet compared to low-starch diet (93.5 vs. 75.6 pM), whereas Cannas et al. (2004) observed the highest IGF-I concentration in Sarda sheep fed a highly digestible fiber.

The IGF-I concentration did not differ between the two species in the mid-lactation morning sampling, but it was significantly and markedly higher in sheep than goats in the sequential after meal short term measurements. On both species, it decreased after the meal, similarly to what observed by Cannas et al. (2004). The higher values observed in ewes are probably associated to their marked insulinemic status and their fast body reserve accumulation, as already explained in the previous paragraph.

Leptin

Leptin was not affected by two diets in both species, according to what already observed by Cannas et al. (2004) in mid lactating sheep or in dairy goats fed a normal or high amylase starch, at equal dietary starch concentrations (Wang et al., 2016). The lack of the effect of the diets could be related to the fact that leptin depends more on the

overall energy balance than by the dietary source, as already observed by Cannas et al. (2004).

The species differences in leptin concentration, with goats having higher values than the ewes, could be linked to differences in the capacity to accumulate body fat reserves. Indeed, in our experiment sheep accumulated much more body reserves than goats, probably because of their lower leptin concentration.

4.4 Overall assessment

The evolution of the metabolites and hormones during the trial and more specifically in mid-lactation suggests that the fact that HS diets stimulated milk production in the goats and body reserves accumulation in sheep, reducing their fat-corrected milk production, was due to the differences observed in the hormonal status of the two species. Indeed, the goats had higher GH concentration than the ewes both during the lactation and also in the sequential after meal assessment, while the ewes had higher insulin, IGF-I and glucose concentrations.

Thus, the ewes had a nutritional status that favored the partition of dietary energy in favor of body reserves accumulation, especially when using the HS diets, which stimulate propionate production, gluconeogenesis and then the action of insulin. The LS diets, in contrast, favored more the production of acetate (Chapter 4), which is not controlled by insulin and then can be used as metabolic fuel, probably sparing glucose and thus favoring milk production and milk fat synthesis (Cannas et al., 2002).

The goats behaved more similarly to dairy cows with high genetic merit, by having higher GH and lower insulin concentrations than the ewes. This probably allowed them to partition, even in mid-lactation, more of the glucose derived from the fermentation of starch for the synthesis of milk rather than for body reserve accumulation.

5. CONCLUSIONS

This research allowed to compare the metabolic and hormonal profiles of dairy ewes and goats under the same feeding ad management conditions. In addition, the metabolic

and hormonal effects of diets differing in starch and digestible fiber concentration was studied.

Overall, it appeared that goats had, throughout the period studied, a hormonal status that favored the partition of dietary energy in favor of milk production, while the ewes showed a hormonal status more prone to stimulate the use of the energy of the diet in favor of body reserves accumulation.

Since no direct effects of the diets on the hormonal status were observed, it is likely that the fact that HS diet stimulated, in comparison to the LS diet, higher milk production in goats and higher body reserve accumulation and loss of milk yield in ewes was due to the different homeorhetic control of energy partitioning during the lactation, more insulinemic in sheep than in goats, especially during mid-lactation. Indeed, the ewes produced more milk fat and fat-corrected milk yield and gained less body reserves by using the LS diets.

This suggests that dairy sheep should be fed, especially after the early lactation stage, with diets having low-starch and high digestible fiber concentration, while goats should receive high-starch diets throughout the lactation.

These species differences in terms of carbohydrate utilization are not obvious. Indeed, often feeding programs for small ruminants are based on indication developed for dairy cows, which might be suitable for dairy goats but certainly not for diary sheep.

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7. TABLES

Table 1. Ingredient and chemical composition of the high-starch (HS) and low-starch (LS) diets supplied during the experiment.

Period	**Early lactation**	**Mid-lactation**	
Groups/Diet	**HS**	**HS**	**LS**
Ingredients (% as fed)			
Pellet (high-starch or low-starch)	65.0	67.0	67.0
Dehydrated chopped alfalfa	32.0	29.0	29.0
Whole corn grain	*	*	*
Mature ryegrass hay	3.0	4.0	4.0
TOTAL	*100.00*	*100.0*	*100.0*
Chemical composition[a]			
DM (% as fed)	88.6	89.6	89.1
CP (% DM)	16.2	15.5	15.6
Ash (% DM)	10.7	11.0	11.2
Ether extract (% DM)	2.3	1.4	1.4
NDF (% DM)	35.4	36.7	48.8
ADF (% DM)	21.5	25.6	35.5
ADL (% DM)	3.6	4.7	5.1
NFC (% DM)[b]	35.5	35.4	23.0
Starch (% DM)	20.4	20.0	7.8

[a] The chemical composition does not include the corn grains supplied at milking; [b] NFC: 100 – CP – ash – NDF - ether extract; * additional supply of whole corn grain: 200 g/d in early lactation and 100 g/d in mid-lactation with the following chemical composition: DM 86.5%, as fed; on a DM basis: CP 8.0%, ash 1.43%, fat 2.1%, NDF 16.7%, ADF 4.7%, ADL 0.9%, NFC 71.8%, starch 69.6%

Table 2. Ingredients and chemical composition of the high-starch and low-starch pellets supplied during the experiment.

Period	**Early lactation**	**Mid-lactation**	
Groups/Pellet	**High-starch**	**High-starch**	**Low-starch**
Ingredients (% as fed)			
Dehydrated alfalfa	30.5	30.0	30.0
Corn meal	26.9	21.1	3.0
Barley meal	13.4	13.4	0.0
Wheat bran	10.1	10.1	5.0
Soybean hulls	-	9.0	43.2
Soybean meal 44	7.9	5.0	7.4
Sugarcane molasses	4.6	4.6	4.6
Sodium bicarbonate	4.3	3.0	3.0
Bentonite	2.0	2.0	2.0
Magnesium oxide	-	1.5	1.5
Minerals and vitamins	0.3	0.3	0.3
Appetizer	0.03	0.03	0.03
TOTAL	100.0	100.0	100.0
Chemical composition			
DM (% as fed)	88.6	90.2	89.3
CP (% DM)	15.2	14.1	14.2
Ash (% DM)	10.7	11.3	11.6
Ether extract (% DM)	2.5	1.4	1.4
NDF (% DM)	27.4	30.7	48.8
ADF (% DM)	14.6	19.9	34.7
ADL (% DM)	1.5	2.9	3.6
NFC (% DM)[a]	44.2	42.6	24.0
Starch (% DM)	30.0	28.1	10.0

[a] NFC: 100 – CP – ash – NDF - ether extract

Table 3. Evolution of plasma glucose concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	**Sheep**	**SEM**[a]	**S**[b]	**P**[c]	**SxP**[d]
Glucose (mg/dl)	**1** (15 ± 5 DIM)[e]	44.98	54.31	2.81			0.062
	2 (21 ± 5 DIM)[f]	49.32	59.25	2.96			0.055
	3 (49 ± 5 DIM)[g]	53.00	58.44	2.28			N.S.[n]
	4 (78 ± 5 DIM)[h]	47.17	51.15	1.85			N.S.
	5 (94 ± 5 DIM)[i]	47.47	46.89	2.65			N.S.
	6 (126 ± 5 DIM)[l]	47.53	54.93	1.77			0.003
	7 (134 ± 5 DIM)[m]	49.01	57.05	2.34			0.045
	Mean	48.35	54.57	1.18	< .0001	<.0001	0.065

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.1$

Table 4. Evolution of plasma non-esterified fatty acids (NEFA) concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	Sheep	SEM[a]	S[b]	P[c]	SxP[d]
NEFA (mmol/L)	**1** (15 ± 5 DIM)[e]	0.63	0.40	0.09			N.S.[n]
	2 (21 ± 5 DIM)[f]	0.48	0.61	0.11			N.S.
	3 (49 ± 5 DIM)[g]	0.36	0.22	0.05			N.S.
	4 (78 ± 5 DIM)[h]	0.32	0.19	0.06			N.S.
	5 (94 ± 5 DIM)[i]	0.09	0.08	0.02			N.S.
	6 (126 ± 5 DIM)[l]	0.15	0.11	0.03			N.S.
	7 (134 ± 5 DIM)[m]	0.17	0.14	0.03			N.S.
	Mean	0.31	0.25	0.03	0.036	<.0001	0.005

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.05$

Table 5. Evolution of plasma urea concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	**Sheep**	**SEM**[a]	**S**[b]	**P**[c]	**SxP**[d]
UREA (mg/dl)	**1** (15 ± 5 DIM)[e]	40.83	31.41	2.69			0.036
	2 (21 ± 5 DIM)[f]	33.18	34.41	2.80			N.S.[n]
	3 (49 ± 5 DIM)[g]	39.91	59.05	2.69			<.0001
	4 (78 ± 5 DIM)[h]	47.31	44.42	2.63			N.S.
	5 (94 ± 5 DIM)[i]	33.56	42.05	2.63			0.08
	6 (126 ± 5 DIM)[l]	46.24	46.97	2.99			N.S.
	7(134 ± 5 DIM)[m]	44.46	44.44	2.63			N.S.
	Mean	40.79	43.25	1.34	0.074	<.0001	<.0001

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.1$

Table 6. Evolution of plasma growth hormone (GH) concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species		*P* level			
		Goats	Sheep	SEM[a]	S[b]	P[c]	SxP[d]
GH (ng/ml)	**1** (15 ± 5 DIM)[e]	4.04	4.11	1.42			N.S.[n]
	2 (21 ± 5 DIM)[f]	5.12	2.78	1.11			N.S.
	3 (49 ± 5 DIM)[g]	11.87	2.81	2.49			0.023
	4 (78 ± 5 DIM)[h]	3.18	1.81	1.01			N.S.
	5 (94 ± 5 DIM)[i]	1.82	1.43	0.41			N.S.
	6 (126 ± 5 DIM)[l]	3.41	1.50	0.83			N.S.
	7 (134 ± 5 DIM)[m]	1.83	1.27	0.34			N.S.
	Mean	4.47	2.28	0.57	0.0004	<.0001	0.012

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h]March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.05$

Table 7. Evolution of plasma insulin concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	Sheep	SEM[a]	S[b]	P[c]	SxP[d]
Insulin (μg/l)	**1** (15 ± 5 DIM)[e]	0.08	0.32	0.06			0.009
	2 (21 ± 5 DIM)[f]	0.11	0.22	0.03			N.S.[n]
	3 (49 ± 5 DIM)[g]	0.07	0.17	0.03			0.036
	4 (78 ± 5 DIM)[h]	0.07	0.14	0.02			0.021
	5 (94 ± 5 DIM)[i]	0.17	0.25	0.03			N.S.
	6 (126 ± 5 DIM)[l]	0.13	0.32	0.05			0.01
	7(134 ± 5 DIM)[m]	0.15	0.44	0.07			0.004
	Mean	0.11	0.26	0.02	<0.0001	<0.0001	0.008

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.05$

Table 8. Evolution of plasma insulin-like growth factor I (IGF-I) concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	Sheep	SEM[a]	S[b]	P[c]	SxP[d]
IGF-I (ng/ml)	**1** (15 ± 5 DIM)[e]	67.01	113.86	22.03			0.039
	2 (21 ± 5 DIM)[f]	81.67	107.26	21.10			N.S.[n]
	3 (49 ± 5 DIM)[g]	73.49	94.74	15.38			N.S.
	4 (78 ± 5 DIM)[h]	117.87	96.97	13.78			N.S.
	5 (94 ± 5 DIM)[i]	117.95	109.23	12.42			N.S.
	6 (126 ± 5 DIM)[l]	100.47	118.18	12.55			N.S.
	7 (134 ± 5 DIM)[m]	101.21	121.17	11.92			N.S.
	Mean	94.24	108.77	11.64	N.S.	0.0005	0.0003

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015; [n] $P>0.05$

Table 9. Evolution of plasma leptin concentration in goats and sheep from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

	Period	Species			*P* level		
		Goats	Sheep	SEM[a]	S[b]	P[c]	SxP[d]
Leptin (ng/ml)	**1** (15 ± 5 DIM)[e]	24.85	10.96	2.70			0.006
	2 (21 ± 5 DIM)[f]	23.65	12.45	2.72			0.004
	3 (49 ± 5 DIM)[g]	30.99	12.05	3.26			<.0001
	4 (78 ± 5 DIM)[h]	30.42	9.42	3.40			<.0001
	5 (94 ± 5 DIM)[i]	27.48	10.89	2.73			<.0001
	6 (126 ± 5 DIM)[l]	23.67	11.99	2.28			<.0001
	7 (134 ± 5 DIM)[m]	25.76	11.93	1.93			<.0001
	Mean	26.26	11.39	2.12	<.0001	0.006	0.0011

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] January 20th, 2015; [f] January 27th, 2015; [g] February 24th, 2015; [h] March 25th, 2015; [i] April 10th, 2015; [l] May 12th, 2015; [m] May 20th, 2015

Table 10. Metabolic and hormonal profile in mid-lactating goats and sheep (from 126 to 134 ± 5 days in milk (DIM); mean ± std. dev; May 12th, 2015 and May 20th, 2015) fed high-starch (HS) and low-starch (LS) diets.

	Species	Diet			*P* level		
		HS	LS	SEM[a]	D[b]	P[c]	DxP[d]
Glucose	Goats	49.16	47.38	2.39	N.S.[h]	N.S.	0.053
(mg/dl)	Sheep	55.66	56.40	2.64	N.S.	N.S.	N.S.
NEFA	Goats	0.15	0.19	0.04	N.S.	N.S.	N.S.
(mmol/L)[e]	Sheep	0.15	0.09	0.02	0.013	0.144	N.S.
UREA	Goats	46.02	43.32	2.70	N.S.	N.S.	N.S.
(mg/dl)	Sheep	45.80	44.75	2.62	N.S.	N.S.	0.057
GH	Goats	2.05	3.18	1.09	N.S.	0.022	N.S.
(ng/ml)[f]	Sheep	1.40	1.38	0.35	N.S.	0.155	N.S.
Insulin	Goats	0.16	0.12	0.03	0.166	N.S.	N.S.
(µg/L)	Sheep	0.34	0.42	0.08	N.S.	0.158	N.S.
IGF-I	Goats	101.01	100.68	22.53	N.S.	N.S.	N.S.
(ng/ml)[g]	Sheep	129.96	109.76	15.03	N.S.	N.S.	0.129
Leptin	Goats	24.67	24.76	3.95	N.S.	0.046	0.109
(ng/ml)	Sheep	11.66	12.28	1.86	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of diet; [c] effect of period; [d] diet x period interaction; [e] non esterified fatty acids; [f] growth hormone; [g] insulin-like growth factor I; [h] $P>0.2$

Table 11. Metabolic and hormonal profile in mid-lactating goats and sheep (from 126 to 134 ± 5 days in milk (DIM); mean ± std. dev; May 12th, 2015 and May 20th, 2015).

	Species			***P* level**		
	Goats	**Sheep**	**SEM**[a]	**S**[b]	**P**[c]	**SxP**[d]
Glucose (mg/dl)	48.3	56.0	1.7	<0.0001	0.122	N.S.[h]
NEFA (mmol/L)[e]	0.16	0.12	0.02	0.098	0.184	N.S.
UREA (mg/dl)	45.2	45.8	2.1	N.S.	N.S.	N.S.
GH (ng/ml)[f]	2.62	1.37	0.58	0.038	0.01	0.041
Insulin (μg/L)	0.14	0.38	0.05	<0.0001	0.069	0.156
IGF-I (ng/ml)[g]	100.8	119.7	13.4	0.167	N.S.	N.S.
Leptin (ng/ml)	24.72	11.97	2.13	<0.0001	0.097	0.076

[a] Standard error of the mean; [b] effect of species; [c] effect of period; [d] species x period interaction; [e] non esterified fatty acids; [f] growth hormone; [g] insulin-like growth factor I; [h] $P>0.2$

8. FIGURES

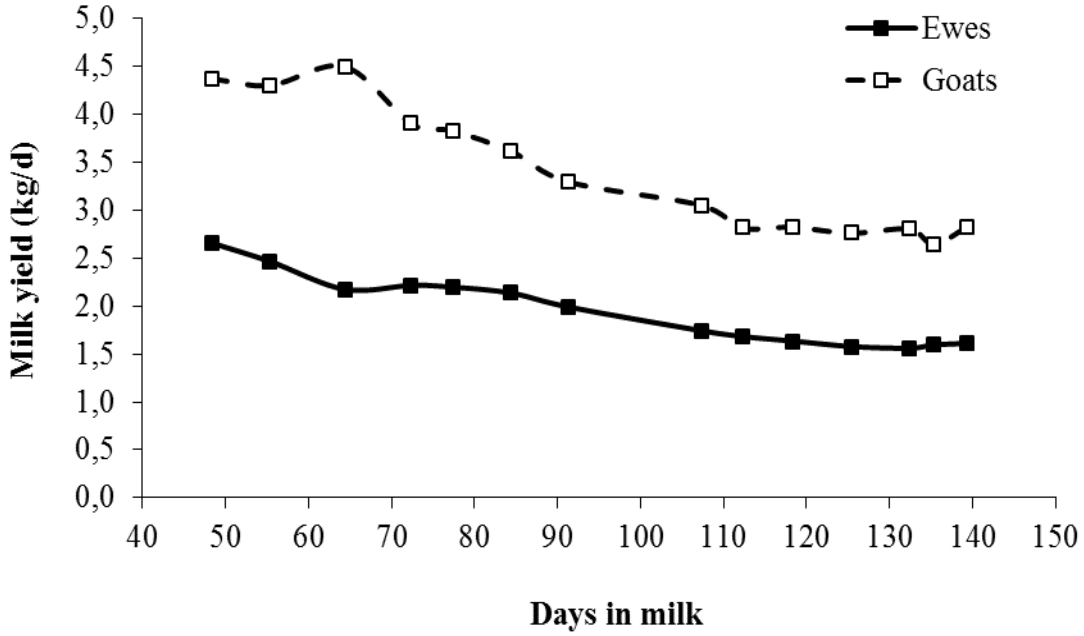

Figure 1. Evolution of milk yield (kg/d) in ewes and goats from early to mid-lactation.

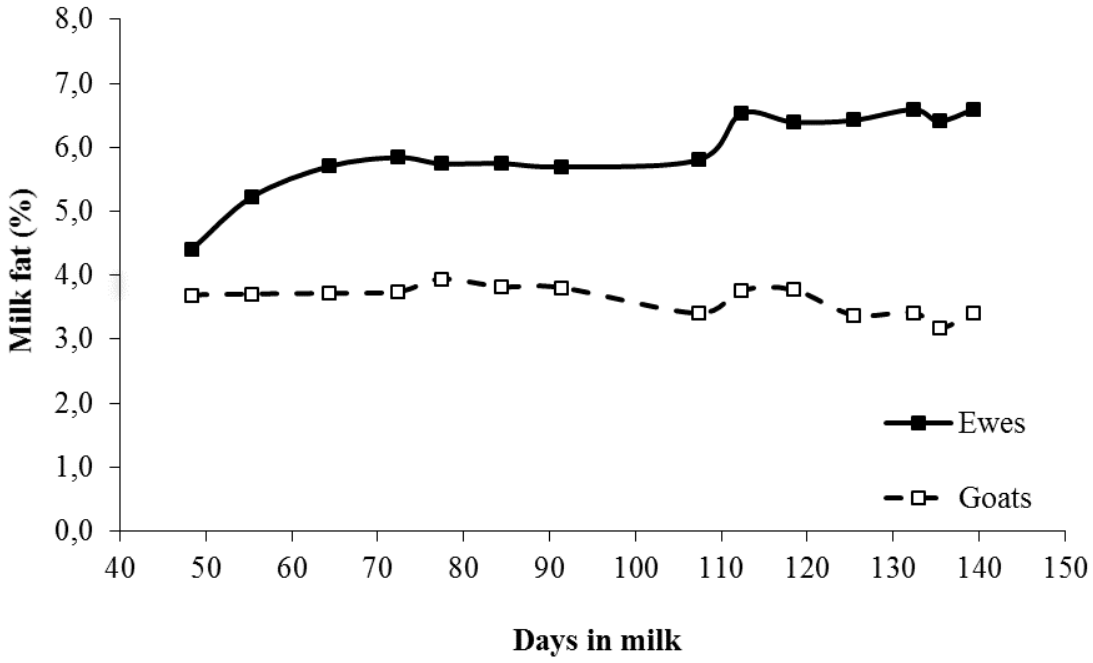

Figure 2. Evolution of milk fat concentration (%) in ewes and goats from early to mid-lactation.

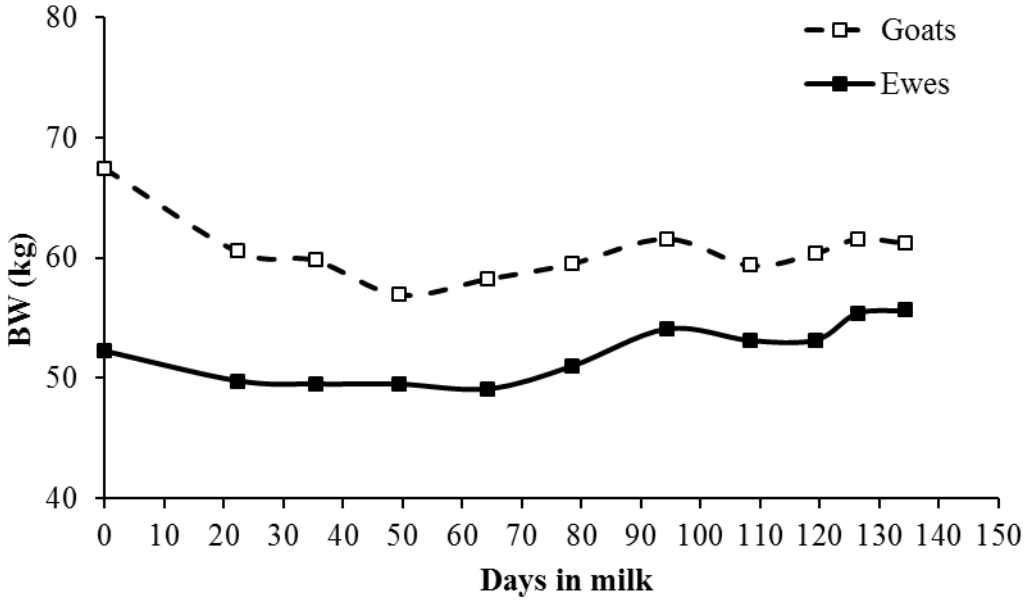

Figure 3. Evolution of body weight (BW; kg) in ewes and goats from early to mid-lactation.

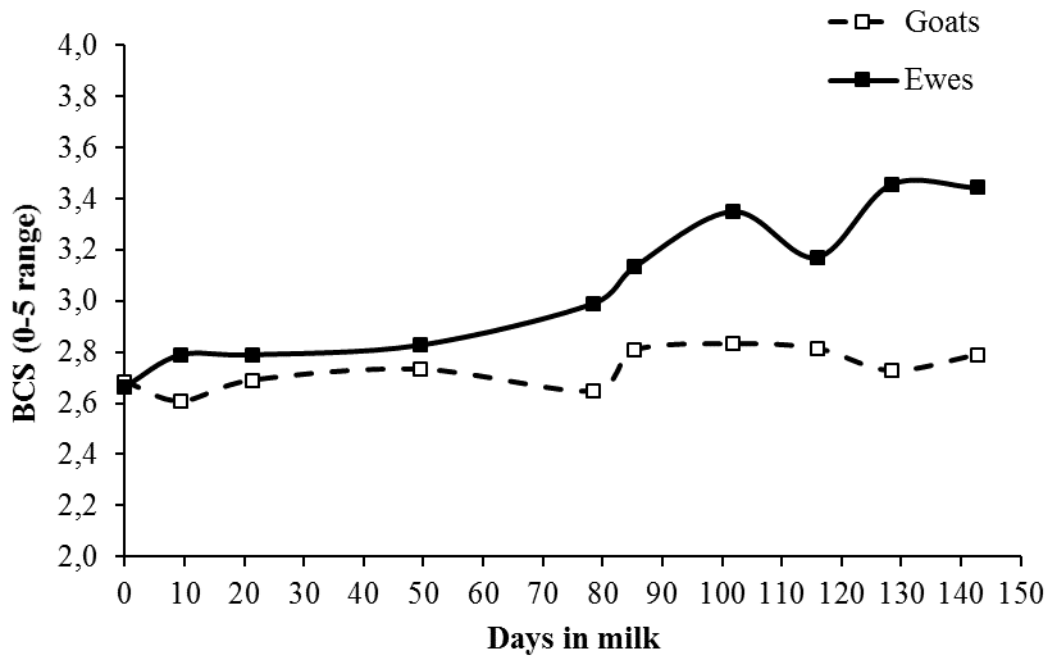

Figure 4. Evolution of body condition score (BCS; 0-5 range) in ewes and goats from early to mid-lactation.

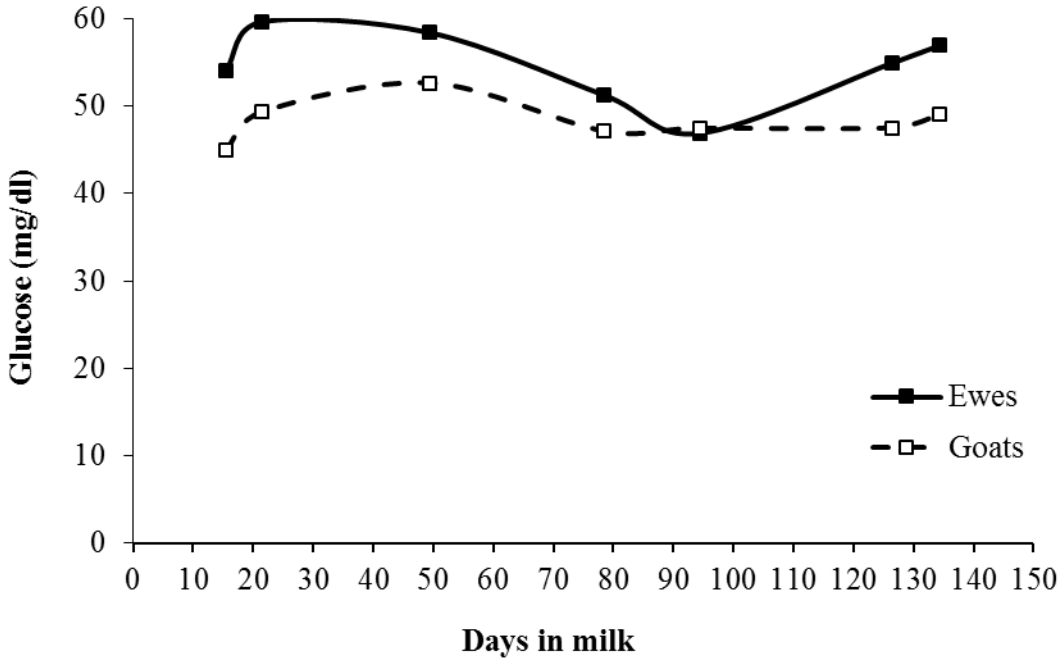

Figure 5. Evolution of plasma glucose concentration (mg/dl) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

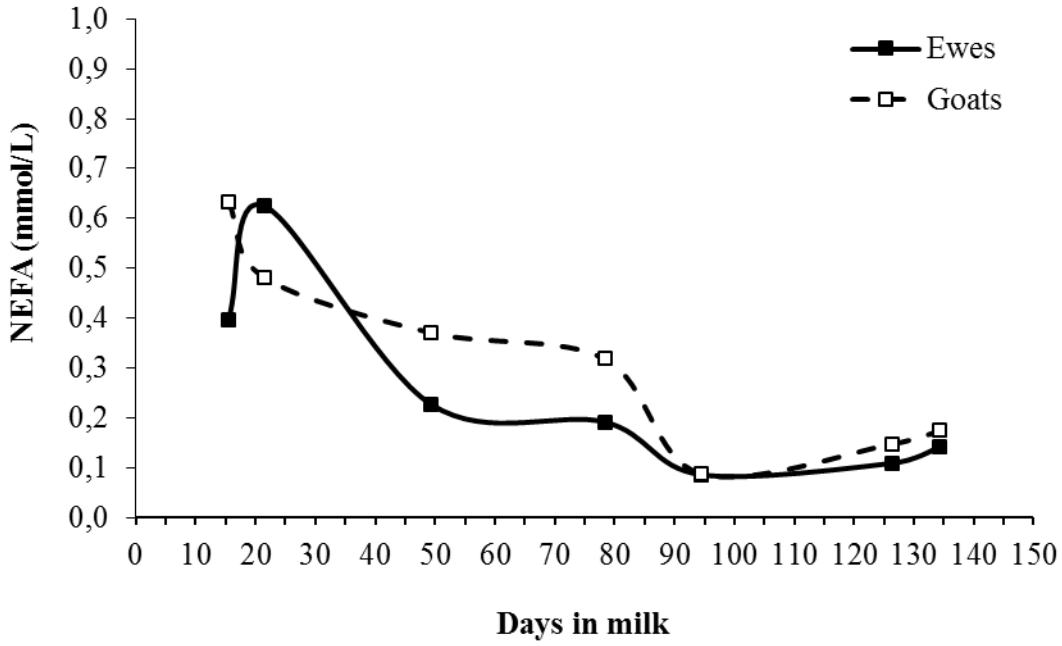

Figure 6. Evolution of plasma non-esterified fatty acids (NEFA) concentration (mmol/L) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

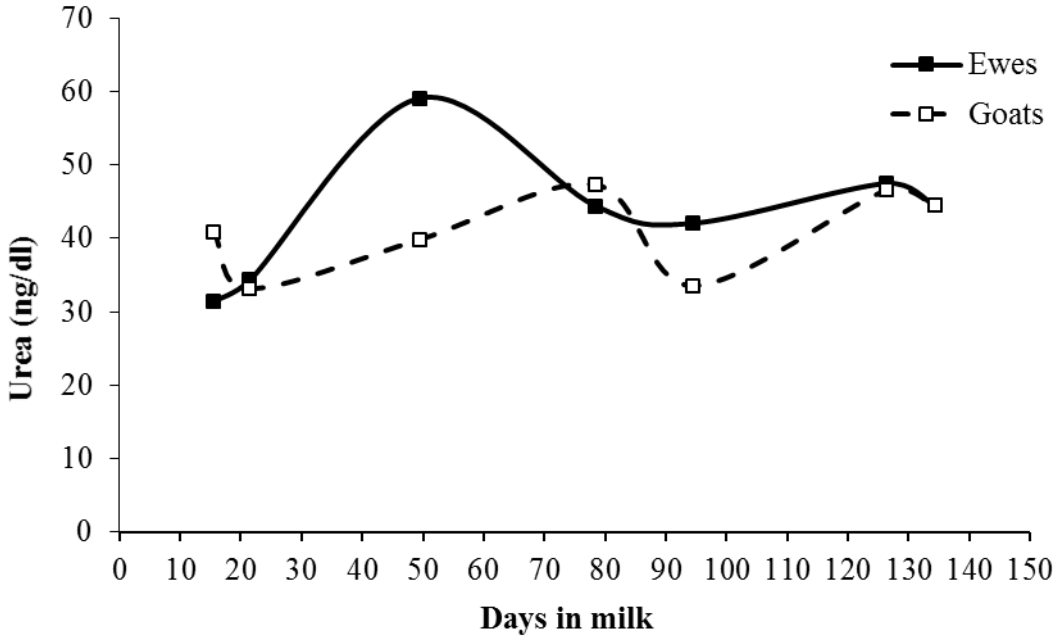

Figure 7. Evolution of plasma urea concentration (mg/dl) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

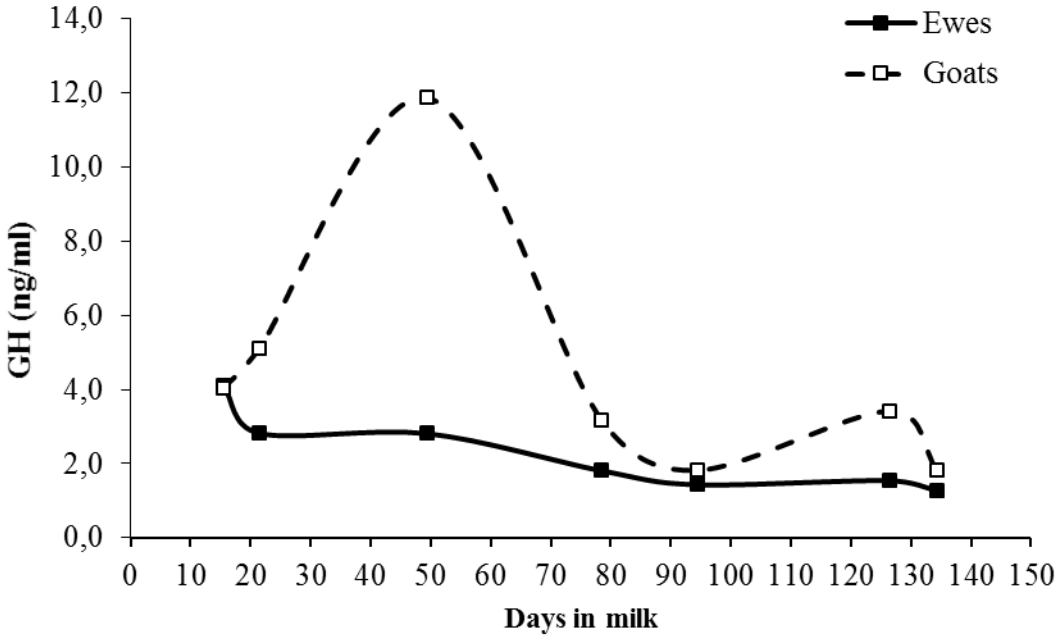

Figure 8. Evolution of plasma growth hormone (GH) concentration (ng/ml) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

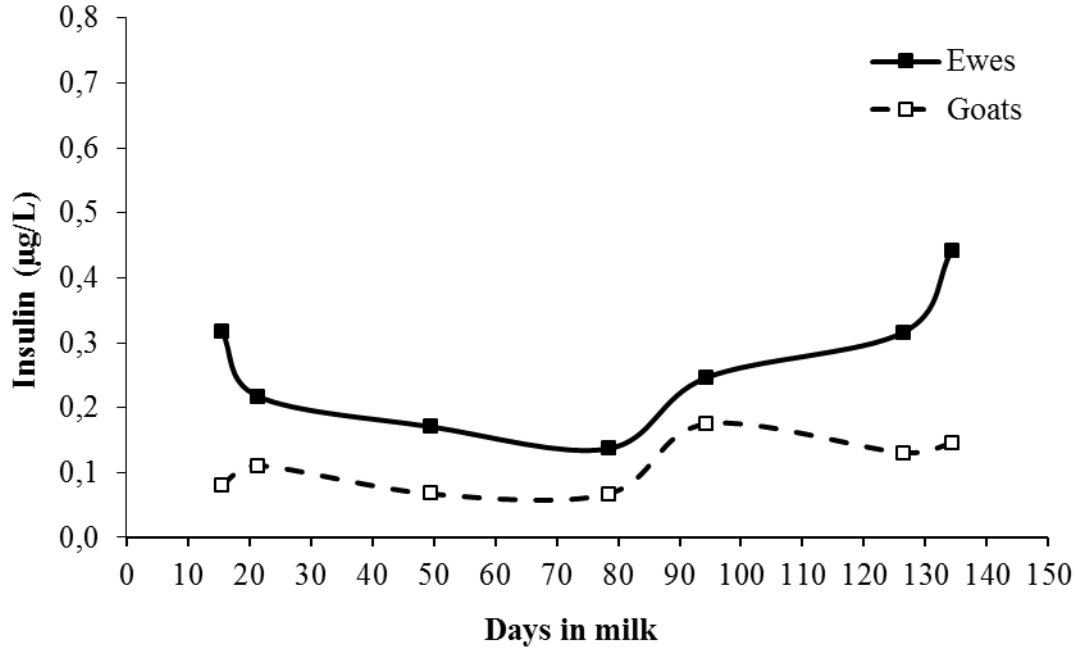

Figure 9. Evolution of plasma insulin concentration (µg/L) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

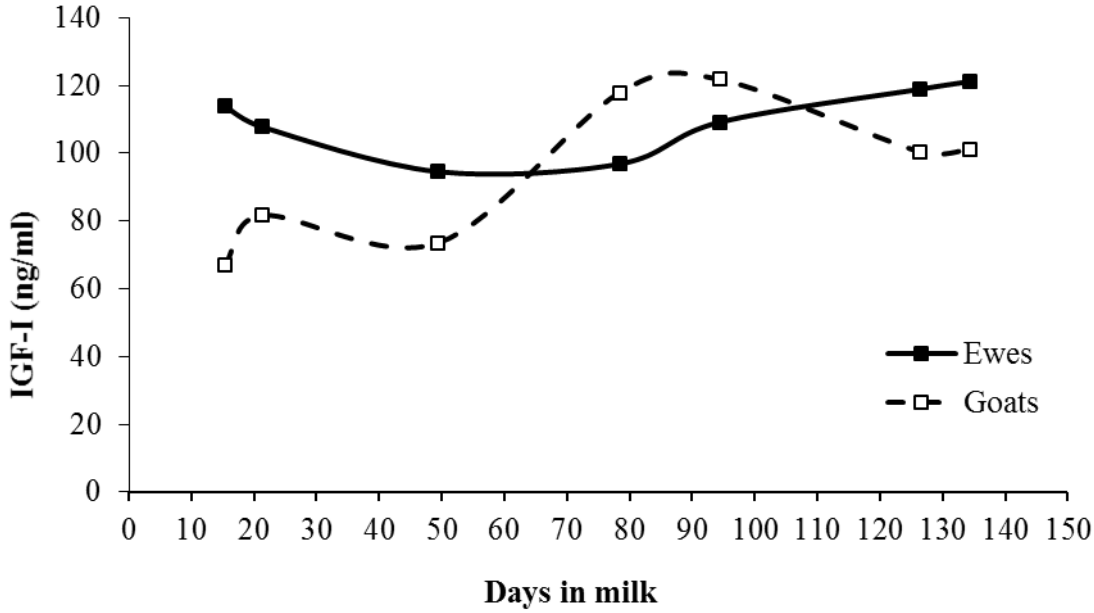

Figure 10. Evolution of plasma insulin-like growth factor I (IGF-I) concentration (ng/ml) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

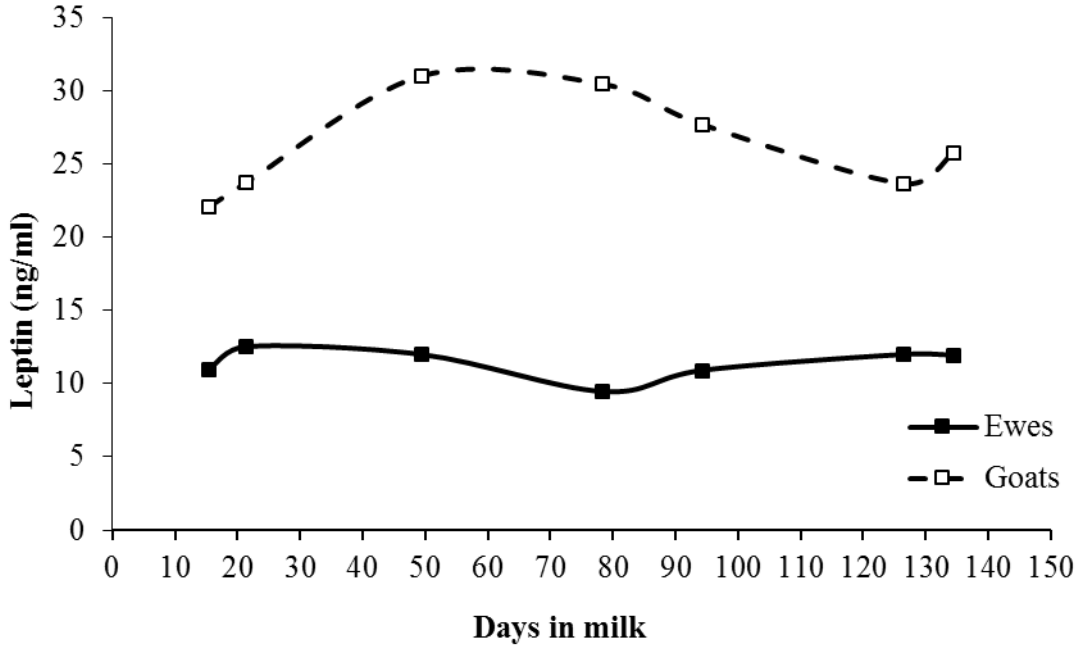

Figure 11. Evolution of plasma leptin concentration (ng/ml) in ewes and goats from early (15 ± 5 days in milk (DIM); mean ± std. dev) to mid-lactation (134 ± 5 DIM).

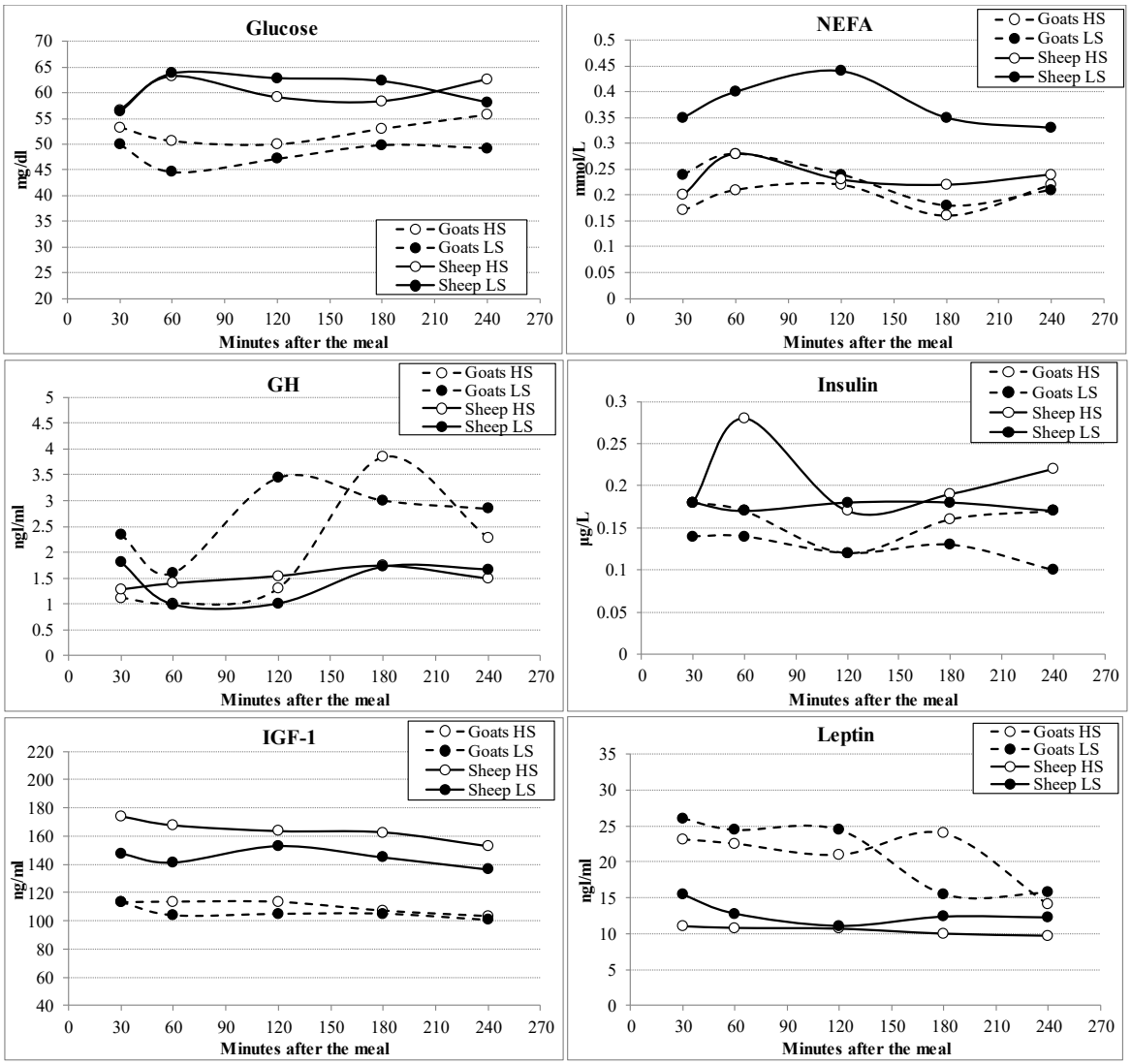

Figure 12. Evolution of metabolites and hormones in the blood sampled at regular intervals after the morning meal at the end of the trial. The effect of diet was always not significant. The effect of species was always significant except for non-esterified fatty acids (NEFA) (Glucose: $P<0.0001$; NEFA: $P>0.05$; growth hormone (GH): $P<0.02$; insulin: $P<0.04$; insulin-like growth factor I (IGF-I): $P<0.002$; Leptin: $P<0.001$). The effect of sampling time was significant for leptin ($P<0.012$) and close to significance for GH ($P< 0.06$).

CHAPTER 4

In-*vivo* digestibility trials on goats and ewes fed high and low-starch diets in early and mid-lactation

ABSTRACT

A series of digestibility trials was carried out to assess possible species differences in the utilization of high-starch (**HS**) diets in early lactation and to quantify the nutritional effects in dairy goats and ewes fed HS and low-starch (**LS**) diets in mid-lactation.

In early lactation, 10 animals of each species were put in individual metabolic cages and fed a HS total mixed ration (20.4% starch, 35.4% NDF, 16.2% CP on DM). The digestibility trials measurements lasted 2 weeks, of which the last 5 days were used for the experimental measurements. From 92 ± 11 DIM a feeding trial was carried out and each species was allocated to two dietary treatments (on DM basis): HS (20.0% starch, 36.7% NDF, 15.5% CP) and LS (7.8% starch, 48.8% NDF, 15.6% CP) diets. At the end of this trial 20 goats and 20 ewes were put in individual metabolic cages and subjected to digestibility measurements, which were carried out in two consecutive cycles in which 20 animals were used (5 goats and 5 ewes with HS diets, 5 goats and 5 ewes with LS diets for each of the two cycles).

During all digestibility trial intake and orts were measured individually and a total collection of feces and urine was carried out for each animal, to assess the digestibility of nutrients, the N balance and the excretion of purine derivatives and thus microbial production. In addition, samples of the rumen fluid were collected.

In early lactation, milk production and dry matter intake (DMI) were higher in goats than in ewes (4.31 vs. 2.23 kg/d; P=0.0001; 3.09 vs. 2.35 kg/d; P=0.051) whereas DMI expressed in % of body weight (BW) did not differ between the two species. NFC apparent digestibility was higher in goats than in ewes (98.46 vs. 91.74%; P<0.0001), whereas dry matter (DM) apparent digestibility and total digestible nutrients (TDN) did not differ between the two species. Total energy requirements were higher in goats than in ewes (7.50 vs. 4.84 Mcal ME_M/d; P=0.0005).

In mid-lactation, milk production was higher in goats than in ewes (P<0.0001) whereas body condition score (BCS) was highest in ewes (P<0.0001) and both did not differ between HS and LS diet. DMI was higher in goats than in ewes both expressed in kg/d (P=0.0005) that in % of BW (P=0.004) and did not vary between the HS and LS diet. DM apparent digestibility did not differ between the two species but it was higher for HS than for LS diet both in goats (68.50 vs. 64.32 %; P<0.0001) that in ewes (67.97 vs. 64.25 %; P<0.0001). NDF true digestibility was higher in goats than in ewes (P<0.0001) and with the LS than the HS diet both in goats (72.67 vs. 68.35 %; P<0.0001) that in ewes (58.77 vs. 52.10 %; P<0.0001). NFC and starch apparent digestibility were higher in ewes than in goats and with HS than the LS diets. TDN did not differ between the two species and was higher in the HS than in the LS diet (goats: 64.35 vs. 60.58; sheep 64.39 vs. 60.59; P<0.0001). Total energy requirements were higher in goats than in ewes (P<0.0001) and did not differ between HS and LS diet.

In early lactation goats had higher rumen pH, ammonia concentration and microbial protein flow than the ewes. When the microbial flow was calculated in proportion of the DMI, there were not species differences. In mid-lactation, the pattern was somehow

similar, even though rumen pH did not differ between the two species and was higher with the LS than the HS diets (P=0.018).
Overall, these results do not suggest any specific nutritional effect in terms of digestibility or rumen fermentations that could explain the productive differences observed in Chapter 2 comparing the two species under two different dietary starch concentrations.

Key words: NFC, NDF, in vivo digestibility, ewes, goats

1. INTRODUCTION

The use of non-fiber carbohydrate (**NFC**) sources, as cereal grains, in early lactation help to increase energy intake reducing the negative effect of energy balance and increasing simultaneously milk production. However, in mid-lactation, NFC affect animal performances differently among small ruminants. The replacement of barley or corn starch with highly digestible fiber sources, such as beet pulps, soybean hulls or immature forages, seems to favor milk persistency in dairy ewes (Cannas et al., 1998; Cannas et al., 2002; Bovera et al., 2004; Cannas et al., 2004; Zenou and Miron, 2005; Cannas et al., 2013) but not in dairy goats. In the latter, instead, the use of high-starch diets in mid and late lactation favors milk production (Cannas et al., 2007; Ibáñez et al., 2015) or does not negatively affect it (Magistrelli et al., 2005; López and Fernández, 2013). These species differences were confirmed in this Thesis, with high-starch diets favoring milk production in dairy goats and body reserve accumulation in dairy ewes (Chapter 2).
These differences could be related to differences in the hormonal control of the partitioning between milk production and body reserves accumulation of the dietary energy derived from carbohydrates. This hypothesis was investigated in Chapter 3, whose results indeed suggest that dairy goats have a hormonal status that favors milk production both in early and mid-lactation, while dairy ewes in the latter stage tend to favor body reserve accumulation.

Other possible reasons for the differences observed between goats and ewes could be related to different abilities to use the high-starch and low-starch diets, both in terms of

total digestibility and in relation to rumen fermentation conditions and stimulation of microbial activity.

Thus, the objective of the research described in this chapter was: i) to compare feed intake and nutrient digestibility of dietary carbohydrates (starch or highly digestible fiber) in sheep and goats during different stages of lactation; ii) to test if the differences in milk production and body reserve accumulation associated to the diets could be explained in terms of diet utilization, rumen fermentation and microbial synthesis.

2. MATERIALS AND METHODS

The experiment was conducted in the experimental farm of AGRIS (Olmedo, Sardinia, Italy), Department of Research on Animal Production.

Two series of digestibility trials on lactating goats and ewes were carried out, one in early lactation and the second one in mid lactation.

2.1 Experimental procedure: animals and diets

Thirty mature Sarda ewes and 30 mature Saanen goats were used for the digestibility trials. The animals were selected from a larger group fed a high-starch diet (Table 1) since parturition (38 sheep and 34 goats), to have groups homogeneous for lambing date, age (6-7 years old) and milk yield.

The study was divided in two periods, early and mid-lactation. In both stages, the animals were kept in metabolic cages for 2 weeks (7 days of adaptation period to the cage and 5 days of measurements). The cages were built in a way that the urines were collected after their excretion in a plastic large container, put below the cage and tilted forward to convey immediately the urines in a bucket, while the feces were collected by a semi rigid plastic net under the cage, tilted backward to convey them in a large rectangular plastic bucket.

Early lactation

Ten ewes (40 ± 3 DIM) and 10 goats (37 ± 4 DIM) were kept in metabolic cages for 2 weeks. They were selected to be representative of their species group in terms of milk yield, body condition score (**BCS**) and lambing date.

All animals were fed the same high-starch diet (**HS**: 20.4% starch, 35.4% NDF, 16.2% CP, on DM; Table 1). The diet, offered as total mixed ration and fed *ad libitum*, contained, on as fed basis, 32.0% of chopped dehydrated alfalfa, 3.0% of mature grass hay, 65.0% of experimental high-starch pellet (Table 2). This pellet was composed (on DM basis) by 27.4% NDF, 44.2% NFC, 30.0% starch and 15.2% CP (Table 2). In addition, 200 g/d of whole corn grains (69.6% starch, 16.7% NDF, 8.0% CP, on DM; Table 1) were supplied during the two daily milkings. At the end of the trial, 8 ewes and 8 goats were slaughtered for experimental purposes, the other were kept in the experimental group for the digestibility trial in mid-lactation.

Mid-lactation

As described in details in Chapter 2, all the remaining animals (30 sheep and 26 goats) were divided in two subgroups for each species, since 92 ± 11 DIM and fed diets rich (HS) or low in starch (**LS**). The two diets were fed *ad libitum* as total mixed rations. They contained (Table 1), on as fed basis, 29.0% of chopped dehydrated alfalfa, 4.0% of mature grass hay and 67.0% of experimental pellet, which differed depending on the groups as follows: i) for the HS groups, a high-starch pellet, with 28.1% starch and 30.7% NDF (on DM basis, Table 2) was used, and ii) for the LS group a low-starch pellet, composed with 10.0% of starch and 48.8% of NDF (on DM basis; Table 2). The pellets differed mainly because most of the corn meal and all the barley meal of HS pellet was replaced by soybean hulls, a high source of highly-digestible fiber, in the LS pellet.

The two diets were iso-proteic (15.5% of crude protein (CP), whereas carbohydrates concentration differed between the two groups: 36.7% NDF, 35.4% NFC and 20.0% starch for the HS diet; and 48.8% NDF, 23.0% NFC, 7.8% starch for the LS diet, on DM basis; Table 1). In addition, 100 g/d of whole corn grains were supplied during milking (69.6% starch, 16.7% NDF, 8.0% CP, on DM; Table 1).

The diets were supplied twice daily (morning and afternoon) after the machine milking (7:00 and 15:00) was completed.

At 140 ± 12 DIM, 10 sheep and 10 goats (for each species, 5 from the HS group, 5 from the LS group) were selected and kept in metabolic cages for 2 weeks for the digestibility measurements. Once these trials were completed, a second run of digestibility trials was carried out. Indeed, at 159 ± 12 DIM other 10 sheep and 10 goats (for each species, 5 from the HS group, 5 from the LS group) were selected and kept in metabolic cages for 2 weeks. For each run the animals were selected to be representative of their species dietary groups in terms of milk yield, BCS and lambing date.
At the end of each run 16 ewes (8 HS and 8 LS) and 16 goats (8 HS and 8 LS) were slaughtered for experimental reasons.

2.2 Measurements and samplings

During the trials the orts were weighted daily during the whole experiment (adaptation and sampling periods) in order to estimate the voluntary feed intake. The amount of the diet offered was adjusted each day considering the orts of the previous day, in order to supply 110% of the amount of diet ingested the previous day.
Samples of the feeds were collected daily, then pooled and subsampled at the end of the trial. Similarly, the daily orts of each animal were collected, pooled, and then subsampled at the end of the trial for the subsequent chemical analysis.
Milk production was monitored as group values during the adaptation period, while during the sampling period it was measured daily and individually at each milking. Individual milk samples taken each milking were collected and immediately stored at 4°C until analysis, which occurred within 1 day from the sampling.
The body weight (BW) was measured once just before putting the animals in the cages and the BCS was measured during the sampling period.
After the adaptation, during the 5 sampling days feces were collected, weighted and then mixed each day, during the experimental period, at 8.00 h and an aliquot (10% of their total fresh weight) was sampled and immediately stored at -20°C until chemical analysis. Various times per day, to avoid accumulation in the floor of the cage or in the

net below it, the feces that did not fall immediately in the bucket were manually removed with a spatula and put in the bucket.

To avoid ammonia losses, the urines produced each sampling day by each animal were collected, just after their excretion in a plastic bucket containing a solution of 20% sulphuric acid in amounts able to keep the pH of the urines below 3. The urines were weighted and mixed each day at 9.00 h and an aliquot was sampled and immediately stored at -20°C until chemical analysis.

Ruminal liquid samples were collected from 16 animals (8 ewes and 8 goats) in early lactation and from 32 animals (16 ewes: 8 HS and 8 LS; 16 goats: 8 HS and 8 LS) in mid lactation from the rumen sac just after they were slaughtered. For the rumen liquid sampling, the whole rumen content was emptied in a bucket and then mixed. A sample of rumen content and of cecum and colon was collected and then filtered with 4 layers of cheesecloth. The pH of the liquid was measured immediately and then the rumen liquid samples were stored immediately at -20 °C.

2.3 Chemical analyses

2.3.1 Feed, orts and feces

The samples of feeds, orts and feces were ground with a Hammer mill by using a 1 mm screen. Before grinding, the fecal samples were thawed and oven-dried at 60°C for 48 h. They were analyzed for DM at 105°C, CP (Kjeldhal method), NDF, ADF, ADL (as reported by Van Soest et al. (1991)), including thermostable α-amilase, ether extract (Soxhlet extraction and method), ash and starch.

The dietary ingredients and the orts rich in starch were pre-treated overnight with 8M urea. The NDF and ADF values were quantified on a ash free basis. Starch was measured with the polarimetric method (Commission Directive 1999/79/EC of 27 July 1999). The NFC were calculated as: 100-CP-NDF-ash-ether extract.

2.3.2 Milk production

Milk samples were analyzed for fat, protein (N x 6.38), lactose (infrared method; Milkoscan 4000, Foss Eletric, HillerØd, Denmark), urea content (enzymatic-

colorimetric method based on Berthelot reaction; Chemspec 150, Bentley Instruments Inc., Chaska, Minnesota, USA) and somatic cell count (**SCC**, flow-cytometry method; Fossomatic 5000, Foss Electric, Hillerød, Denmark). Daily milk production and composition were obtained through the weighted mean between the morning and afternoon production and composition data.

Fat-corrected milk (**FCM**) yield was calculated separately for the two species. For ewes, milk production was normalized at 6.5% fat as $FCM_{(6.5\%)}$ = 0.37 × milk yield (kg/d) + 9.7 × milk fat (%) × milk yield (kg/d), according to the equation developed by Pulina et al. (1989). For goats, milk production was normalized at 3.5% fat as $FCM_{(3.5\%)}$ = 0.63 × milk yield (kg/d) + 10.5 × milk fat (%) × milk yield (kg/d), according to Pulina et al. (1992).

Milk energy content was calculated separately for the two species. Net energy for lactation (NE_L, Mcal NE/d) was calculated as NE_L = (251.73 + 89.64 × PQ + 37.85 × (PP/0.95)) × Yn/1000 for ewes, and NE_L = (289.72 + 71.93 × PQ + 48.28 × (PP/0.92)) × Yn/1000 for goats, according to Tedeschi et al. (2010). In particular, Yn is measured milk yield at a particular day of lactation (kg/d), PQ is measured milk fat at a particular day of lactation (%), PP is measured true milk protein for a particular day of lactation (%).

2.3.3 Urines

Daily urine individual samples collected during all of the experimental days were mixed in proportion to daily urines individual production to obtain an individual sample per animal. Urine samples were analyzed for total N (Kjeldhal method) and for purine derivatives (allantoin, ipoxhantin, xanthin, and uric acid).

For purine derivatives, urine samples were diluted in 1:100 ratio for allantoin, ipoxhantin and uric acid analyses and in 1:10 ratio for xanthin determination, then they were filtered.

Purine derivatives were quantified by using two Spherisorb ODS-5 columns (250 mm x 4.16 mm I.D.) connected with a $NH_4H_2PO_4$-$NH_4H_2PO_4$-acetonitrile (80:20) gradient, monitoring the effluent at 205 nm according to Balcells et al. (1992).

Purine derivatives were used in order to estimated microbial N, whereas total N was used in order to estimate the N balance of each animal.

For both species, absorbed purine (mmol/day) were estimated using the Chen and Gomes (1992) equation:

$$Y=0.84\ X + 0.150\ BW^{0.75}\ e^{-0.25x}$$

where Y is the excreted purine derivates in urine (mmol/d), x is the amount of absorbed purine (mmol/d), 0.84 is the recovery of absorbed purine in the urine, $BW^{0.75}$ is the metabolic BW (kg) and $0.150\ BW^{0.75}\ e^{-0.25x}$ is the correction for the contribution of endogenous purines.
The amount of microbial N was obtained as:

$$\text{Microbial N (g N/d)} = (X\ (\text{mmol/d}) \times 70) /0.116 \times 0.83 \times 1000 = 0.727 \times X$$

where 0.83 is the digestibility coefficient of microbial purine (83%), 70 is the amount of N in the purine (70 mg of N/mmol), whereas the purine N/total N ratio of microbial population is 11.6/100.

The N balance was estimated as: feed N intake (g/d) - fecal N (g/d) - urine N (g/d) - milk N (g/d).

2.3.4 Ruminal liquid analysis

The samples of ruminal liquid were filtered, the pH value was measured and the samples were stored at -20°C until the analysis for ammonia and volatile fatty acid (VFA) could be carried out. Ammonia was determined through a colorimetric method (as reported by Chaney and Marbach (1962), but using salicylate instead of phenol), by using a UV visible spectrophotomer (Varian, Inc., Palo Alto, CA, USA).
Volatile fatty acids (acetic, propionic, butyric, iso-butyrric, valerianic, iso-valerianic) were analyzed by using a high-performance liquid chromatography method (HPLC; Varian Inc., Palo Alto, California, USA).

2.4 Digestibility calculations

Individual voluntary dietary intake and its composition was estimated as the difference between diet offered and orts, correcting for the chemical composition of the orts of each animal. Intake was expressed in absolute terms (kg/d) and as proportion of BW.

The digestibility coefficients of DM and of each chemical component of the diet were calculated as:

Digestibility (%) = ((nutrient intake - nutrient excreted)/nutrient intake) * 100

The dietary energy concentration was estimated by using the digestibility coefficients found in this experiment as proposed by Van Soest to estimate the total digestible nutrients: *TDN= digestible DM + 1.25 x digestible EE – total ash* (Eq. 25.3; Van Soest, 1994). The conversion of TDN into NE of lactation (NEL) was calculated as follows: *DE (Mcal/kg of DM) = TDN/100×4.409*; *ME (Mcal/kg of DM) = 0.82×DE*; *NEL (Mcal/kg ofDM) = 0.644×ME* (Cannas et al., 2004).

2.5 Statistical analysis

Data on milk production, BW, BCS, feed intake, digestibility coefficient, energetic dietary value, microbial protein, N balance, pH, VFA, and ammonia were analyzed by the PROC GLM procedure of SAS (Version 9.0, SAS Institute Inc., Cary, NC, USA). In early lactation, a linear model was used to test the differences between the species as reported in the following model:

$$Y_i=\mu + \alpha_i + \varepsilon_i$$

where Y_i is the dependent variable, μ is the general mean, α_i is the effect of species (i = sheep, goats) and ε_i is the residual error.

In mid lactation, the same model was used to test the differences between the species, diets, periods (*i.e*. digestibility cycles) and their interactions as reported in the following model:

$$Y_{ijk}=\mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the general mean, α_i is the effect of species (i=sheep, goats), β_j is the effect of diet (j= HS, LS), γ_k is the effect of period (k=1, 2), $\alpha\beta_{ij}$ is the species x diet interaction (i=sheep, goats; j= HS, LS), $\alpha\gamma_{ik}$ is the species x period interaction (i=sheep, goats; k=1, 2), $\beta\gamma_{jk}$ is the diet x period interaction (j=HS, LS; k=1, 2) and ε_{ijk} is the residual error.

In the mid lactation digestibility trials, 2 animals (1 sheep and 1 goat) with very low voluntary feed intake and milk yield were not considered in the statistical analysis.
Data were expressed as mean ± SEM. Means were separated using Tukey's test. The accepted level of significance was $P<0.05$.

3. RESULTS

3.1 Early lactation

Milk production

Milk yield was higher in goats than in ewes (4.31 vs. 2.23 kg/d ± 0.43 (mean ± SEM); P=0.0001) (Table 3). FCM and NE_L were also higher in goats than in ewes (Table 3). Milk fat concentration (Table 3) was higher in ewes than in goats (4.83 vs. 3.80 % ± 0.21; P=0.0001), whereas milk fat yield was highest in goats (163.04 vs. 107.05 g/d ± 19.64; goats vs. ewes; respectively; P=0.011) (Table 3). Milk protein concentration (Table 3) was higher in ewes than in goats (4.45 vs. 3.07 % ± 0.16; P<0.0001), whereas milk protein yield was highest in goats (132.37 vs. 98.10 g/d ± 14.61; goats vs. ewes; respectively; P=0.031) (Table 3). Milk lactose concentration (Table 3) did not differ between the two species, whereas milk lactose yield was higher in goats than in ewes (201.61 vs. 108.59 g/d ± 19.34; P=0.0001) (Table 3). Urea and somatic cell count (**SCC**) did not differ between the two species (Table 3).

Body reserves

The BW was higher in goats than in ewes (57.81 vs. 47.41 kg ± 2.99; P=0.003) (Table 3), whereas BCS did not differ between the two species (Table 3).

Intake (expressed in kg/d)

Intake (as fed) was higher in goats than in ewes (3.48 vs. 2.64 kg/d ± 0.40; P=0.049) (Table 4). DM intake (DMI) was higher in goats than in ewes (3.09 vs. 2.35 kg/d ± 0.36; P=0.051) (Table 4). CP intake was higher in goats than in ewes (0.49 vs. 0.36 kg/d ± 0.06; P=0.045) (Table 4). NDF intake was higher in goats than in ewes (1.03 vs. 0.78 kg/d ± 0.12; P=0.046) (Table 4). NFC and starch intake did not differ between the two

species even though was numerically greater in goats than in ewes (1.19 vs. 0.92 ± 0.13; 0.74 vs. 0.58 kg/d ± 0.08) (Table 4).

Level of intake (expressed as % of BW)

Neither DM or its chemical constituents were different between the two species when expressed as proportion of BW (Table 5).

Digestibility

The in *vivo* digestibility coefficients (expressed in % on DM) (Table 6) did not differ between the two species, except for NFC apparent digestibility that was higher in goats than in ewes (98.46 vs. 91.74 % ± 0.35; P<0.0001) (Table 6). TDN did not differ between the two species (Table 6).

Digested DM intake was higher in goats than in ewes (2.13 vs. 1.62 kg/d ± 0.23; P=0.042) (Table 7). Digested CP intake was higher in goats than in ewes (0.34 vs. 0.26 kg/d ± 0.04; P=0.034) (Table 7). Digested NDF intake higher in goats than in ewes (0.48 vs. 0.37 kg/d ± 0.05; P=0.043) (Table 7). Digested NFC intake was higher in goats than in ewes (1.17 vs. 0.84 kg/d ± 0.13; P=0.021) (Table 7). Digested starch intake did not differ between the two species even tough was numerically greater in goats than in ewes (0.72 vs. 0.57 kg/d ± 0.08) (Table 7).

Energetic value, requirements and energy balance

DE, ME and NEL did not differ between the two species (Table 8). Metabolizable energy intake (MEI) was higher in goats than in ewes (7.56 vs. 5.59 Mcal ME/d ± 0.83; P=0.03) (Table 8). Similarly, ME required for maintenance (ME_M) was higher in goats than in ewes (2.67 vs. 1.87 Mcal ME_M/d ± 0.13; P<0.0001) (Table 8). The ME for lactation (ME_L) was higher in goats than in ewes (4.84 vs. 2.97 Mcal ME_L /d ± 0.51; P=0.0019) (Table 8). Total energy requirements (in terms of ME) were higher in goats than in ewes (7.50 vs. 4.84 Mcal ME/d ± 0.62; P=0.0005) (Table 8). The energy balance (EB) was more positive in ewes than in goats (0.75 vs. 0.06 ± 0.35; P=0.069) (Table 8).

Rumen VFA and NH_3

Rumen and gross intestine pH was higher in the goats than in the ewes, even though the P values were slightly above the 0.05 threshold (Table 9). The NH_3 concentration was markedly higher in goats than in ewes (23.38 vs. 11.89 mg/dl ± 3.46; P=0.005), whereas none of the volatile fatty acids differed between the two species (P>0.05) (Table 9).

Microbial protein and N balance

Microbial protein flow was higher in goats than in ewes (130.64 vs. 90.72 g CP/d ± 14.62; P=0.0137), whereas N balance did not differ between the two species (11.49 vs. 9.26 g/d ± 2.84; goats vs. ewes; respectively) (Table 10).

3.2 Mid-lactation

Milk production

Effect of species

Milk yield, FCM and NE_L were higher in goats than in ewes (P<0.0001) (Table 11). Milk fat concentration was higher in ewes than in goats (P<0.0001), whereas milk fat yield did not differ between the two species (Table 11). Milk protein concentration (Table 11) was higher in ewes than in goats (P<0.0001), whereas milk protein yield was highest in goats (P<0.0001) (Table 11). Milk lactose concentration (Table 11) did not differ between the two species, whereas milk lactose yield was higher in goat than in ewes (P<0.0001) (Table 11). Milk urea (Table 11) was higher in goats than in ewes (P<0.0001), whereas SCC did not differ between the two species (Table 11).

Effect of diet

The effect of diet was not statistically significant for any of the milk parameters considered except for milk urea (P=0.041), that in goats was higher in the LS than in the HS diet (41.24 vs. 34.73 mg/dl) (Table 11).

Effect of period (digestibility cycle)

The effect of period was statistically significant for milk yield (P=0.0004), FCM (P=0.0009), NE_L (P=0.0017), milk fat concentration (P=0.009), milk fat yield

(P=0.0043), milk protein (P=0.012), milk protein yield (P=0.0017), milk lactose yield (P=0.0013), whereas it was not statistically significant for milk lactose concentration, milk urea and SCC (Table 11).

Effect of species x diet interaction

The effect of species x diet interaction was statistically significant only for milk urea (P= 0.014) (Table 11).

Effect of species x period interaction

The effect of species x period interaction was statistically significant for milk yield (P=0.024), FCM (P=0.004), NE_L (P=0.009), milk fat concentration (P<0.0001), milk fat yield (P=0.004), milk protein yield (P=0.049), milk lactose yield (P=0.03), milk urea (P=0.013), whereas it was not statistically significant for milk protein concentration, milk lactose concentration and SCC (Table 11).

Effect of diet x period interaction

The effect of diet x period interaction was not significant for any of the milk parameters considered (Table 11).

Body reserves

Effect of species

BW was higher in goats than in ewes (P=0.013), whereas BCS was highest in ewes (P<0.0001) (Table 11).

Effect of diet

The effect of diet was not statistically significant for BW and BCS (Table 11).

Effect of period

The effect was not statistically significant for BW and BCS (Table 11).

Effect of species x diet interaction

The effect of species x diet interaction was not statistically significant for BW and BCS (Table 11).

Effect of species x period interaction

The effect of species x period interaction was not statistically significant for BW and BCS (Table 11).

Effect of diet x period interaction

The effect of diet x period interaction was not statistically significant for BW and BCS (Table 11).

Intake (expressed in kg/d)

Effect of species

Intake (as fed) (P=0.0005), DMI (P=0.0005), ash intake (P=0.0004), OM intake (P=0.0004), fat intake (P=0.0011), CP intake (P=0.0002), NDF intake (P=0.0012), ADF intake (P=0.0007), ADL intake (P=0.0001), NFC intake (P=0.0003) and starch intake (P=0.0007) were higher in goats than in ewes (Table 12).

Effect of diet

The effect of diet was not statistically significant for any of the intake parameters considered, except for NFC and starch intake that were higher in the HS than in the LS diet (P<0.0001) (Table 12).

Effect of period

The effect of period was not statistically significant for any of the intake parameters considered except for starch intake (P=0.02) (Table 12).

Effect of species x diet interaction

The effect of species x diet interaction was not statistically significant for any of the intake parameters considered (Table 12).

Effect of species x period interaction

The effect of species x period interaction was not statistically significant for any of the intake parameters considered (Table 12).

Effect of diet x period interaction

The effect of diet x period interaction was not statistically significant for any of the intake parameters considered (Table 12).

Intake (expressed in % of BW)

Effect of species

The level of intake of DM and all its chemical constituents, were higher in goats than in ewes (Table 13).

Effect of diet

The effect of diet was not statistically significant for any of the level of intake values considered, except for NFC and starch intake that were higher in the HS than in the LS diet ($P<0.0001$) (Table 13).

Effect of period

The effect of period was not statistically significant for any of the intake parameters considered (Table 13).

Effect of species x diet interaction

The effect of species x diet interaction was not statistically significant for any of the intake parameters considered (Table 13).

Effect of species x period interaction

The effect of species x period interaction was not statistically significant for any of the intake parameters considered (Table 13).

Effect of diet x period interaction

The effect of diet x period interaction was not statistically significant for any of the intake parameters considered (Table 13).

Digestibility

Effect of species

DM, ash, OM, and fat apparent digestibility did not differ between the two species (Table 14). CP apparent digestibility (P=0.002) (Table 14) and NDF true digestibility (P<0.0001) were higher in goats than in ewes (Table 14), whereas ADF true digestibility did not differ between the two species (Table 14). NFC apparent digestibility (P<0.0001) and starch apparent digestibility (P=0.006) were higher in ewes than in goats (Table 14), whereas TDN did not differ between the two species (Table 14).

Digested DM intake (P=0.0002), digested ash intake (P<0.0001), digestible OM intake (P=0.0002), digested fat intake (P=0.008), digested CP intake (P<0.0001), digested NDF intake (P<0.0001) and digested ADF intake (P=0.0004) were higher in goats than in ewes (Table 15). Digested NFC intake did not differ between the two species (Table 15), whereas digested starch intake was higher in goats than in ewes (P=0.001) (Table 15).

Effect of diet

DM apparent digestibility was higher in the HS than in the LS diet (P<0.0001) (Table 14). Ash apparent digestibility did not differ between the two diets (Table 14). OM, fat and CP apparent digestibility were higher in the HS than in the LS diet (P<0.0001) (Table 14). NDF and ADF true digestibility were higher in the LS than in the HS diet (P<0.0001) (Table 14). NFC (P<0.0001) and starch (P=0.001) apparent digestibility were higher in the HS than in the LS diet (Table 14). TDN was higher in the HS than in the LS diet (P<0.0001) (Table 14).

Digested DM, ash, OM, fat, CP, and NDF intake did not vary between the two diets (Table 15). Digested ADF intake was higher in the LS than in the HS diet (P=0.012),

whereas digested NFC and starch intake were higher in the HS than in the LS diet (P<0.0001) (Table 15).

Effect of period

The effect of period was statistically significant for fat apparent digestibility (P<0.0001) (Table 14), CP apparent digestibility (P=0.02) Table 14), NDF true digestibility (P<0.0001) (Table 14), ADF true digestibility (P=0.004) (Table 14), NFC apparent digestibility (P<0.0001) (Table 14), digested NDF intake (P=0.003) (Table 15), digested NFC intake (P=0.020) (Table 15) and digested starch intake (P=0.012) (Table 15) whereas was not statistically significant for DM, ash, OM and starch apparent digestibility (Table 14), TDN (Table 14), digested DM, ash, OM, fat, CP and ADF intake (Table 15).

Effect of species x diet interaction

The effect of species x diet interaction was not statistically significant for any of the parameters considered except for NFC apparent digestibility (P=0.008) (Table 14).

Effect of species x period interaction

The effect of species x period interaction was statistically significant for fat apparent digestibility (P=0.004) (Table 14), CP apparent digestibility (P=0.038) (Table 14), NDF true digestibility (P<0.0001) (Table 14), ADF true digestibility (P=0.024), NFC apparent digestibility (P<0.0001) (Table 14), digested NDF intake (P=0.014) (Table 15) and for digested NFC intake (P=0.003) (Table 15) whereas was not statistically significant for DM, ash, OM and starch apparent digestibility (Table 14), TDN (Table 14), digested DM, ash, OM, fat, CP, ADF and starch intake (Table 15).

Effect of diet x period interaction

The effect of diet x period interaction was statistically significant for DM apparent digestibility (P=0.01) (Table 14), OM apparent digestibility (P=0.007) (Table 14), fat apparent digestibility (P=0.009) (Table 14), CP apparent digestibility (P=0.001) (Table 14), NFC apparent digestibility (P=0.002) (Table 14), and for TDN (P=0.004) (Table 14) whereas was not statistically significant for ash apparent digestibility (Table 14),

NDF and ADF true digestibility (Table 14), starch apparent digestibility (Table 14), digested DM intake (Table 15), digested ash intake (Table 15), digested OM intake (Table 15), digested fat intake (Table 15), digested CP intake (Table 15), digested NDF intake (Table 15), digested ADF intake (Table 15), digested NFC intake (Table 15), and for digested starch intake (Table 15).

Energetic value, requirements and energy balance

Effect of species

DE, ME and NEL concentrations of the diets did not differ between the two species (Table 16). The MEI was higher in goats than in ewes (P=0.0002) (Table 16). The ME_M and ME_L and thus total ME energy requirements were higher in goats than in ewes (P<0.0001) (Table 16). The energy balance (EB) did not differ between the two species (Table 16).

Effect of diet

The DE, ME and NEL concentrations were higher in the HS than in the LS diet (P<0.0001) (Table 16). MEI did not differ between the two diets (Table 16). ME_M, ME_L, and total energy requirements did not differ between the two diets (Table 16). EB was more negative for the LS than for the HS diet (P=0.02) (Table 16).

Effect of period

The effect of period was not statistically significant for the parameters considered, except for ME_L (P=0.02) and for total ME requirements (P=0.003) (Table 16).

Effect of species x diet interaction

The effect of species x diet interaction was not statistically significant for the parameters considered (Table 16).

Effect of species x period interaction

The effect of species x period interaction was not statistically significant for the parameters considered, except for MEL (P=0.01) and for total energy requirements (P=0.03) (Table 16).

Effect of diet x period interaction

The effect of diet x period interaction was not statistically significant except for DE (P= 0.005), ME (P=0.006) and for NEL (P=0.004) (Table 16).

Rumen fermentation

Effect of species

Rumen pH did not differ between species, while cecum and colon pH was higher in the goats than in the ewes (P<0.0015; Table 17).

The concentration of NH_3 in the rumen was higher in goats than in ewes (P=0.004) (Table 17). Rumen acetic acid expressed in mMol/L did not differ between the two species, whereas acetic acid expressed in % was higher in goats than in ewes (P=0.005) (Table 17). Propionic acid expressed (in mMol and in %), butyric acid (expressed in mMol and in %), iso-valerianic acid, and valerianic acid not differ between the two species (Table 17). Iso-butyric acid was higher in ewes than in goats (P=0.018) (Table 17).

Effect of diet

The rumen pH values were higher in the LS diet compared to the HS diet (P<0.018), while did not differ in the gross intestine as effect of the diets used (Table 17).

NH_3 did not vary between HS and LS diets (Table 17). Acetic acid, expressed in mMol did not differ between the two diets, whereas its molar percentage was higher in the LS than in the HS diet (P=0.001) (Table 17). Propionic acid (expressed in mMol and in %) and iso-butyric did not differ between the two diets, whereas butyric acid (expressed in mMol and in %) was higher in the HS than in the LS diet (Table 17). Iso-valerianic was higher in the HS than in the LS diet (P=0.007), whereas valerianic acid did not differ between the two diets (Table 17).

Effect of period

The effect of period was statistically significant for acetic acid (mMol) (P=0.021), acetic acid (%) (P=0.002), propionic acid (P=0.033) (mMol), iso-butyric acid (P=0.0006), butyric acid (mMol) (P<0.0001), butyric acid (%) (P=0.023), iso-valerianic acid (P<0.0001) and for valerianic acid (P=0.0001), whereas it was not statistically significant for NH_3 and propionic acid (%) (Table 17).

Effect of species x diet interaction

The effect of species x diet interaction was statistically significant only for butyric acid (mMol) (P=0.012), iso-valerianic acid (P=0.032) and for valerianic acid (P=0.0006) (Table 17).

Effect of species x period interaction

The effect of species x period interaction was not statistically significant for all of the parameters considered except for cecum and colon pH (P=0.015) (Table 17).

Effect of diet x period interaction

The effect of diet x period interaction was statistically significant only for butyric acid (P=0.002) and for iso-valerianic acid (P=0.0008) (Table 17).

Microbial Protein and N balance

Effect of species

Microbial N production was higher in goats than in ewes (P<0.0001), whereas N balance did not differ between the two species (Table 18).

Effect of diet

Microbial N did not differ between the two diets whereas microbial protein /kg NDFI was higher in the HS than in the LS diet (P=0.028). N balance was higher for the HS than the LS diet (P=0.039) (Table 18).

Effect of period

The effect of period was statistically significant only for microbial N (P=0.037) (Table 18).

Effect of species x diet interaction

The effect of *species x diet interaction* was not statistically significant for the two parameters considered (Table 18).

Effect of species x period interaction

The effect of *species x period interaction* was not statistically significant for the two parameters considered (Table 18).

Effect of diet x period interaction

The effect of *diet x period interaction* was not statistically significant for the two parameters considered (Table 18).

4. DISCUSSION

This study was carried out to compare the voluntary feed intake, digestibility, rumen fermentations, and energy balance of ewes and goats fed using the same high-starch diet in early lactation and two different diets in mid-lactation, one high in starch and the other one low in starch and high in digestible fiber.

The main objective of all the research was to compare goats and sheep in mid-lactation and to test possible effects of the diet on their hormonal status, lactation persistency and on the partitioning of dietary energy. The digestibility trial in early lactation was planned to assess if there were species differences in early lactation in dietary utilization (This Chapter) and if they could be associated to the hormonal status of the animals in the same stage (Chapter 3).

Three in *vivo* digestibility cycles were carried out: one in early lactation and two in mid-lactation.

4.1 Early lactation

Milk production and body reserves

As planned and based on the well-known productive difference between dairy goats and dairy sheep, milk production was much higher in the goats than in the ewes, despite the ewes were chosen for their high genetic merit. The ewes had, only numerically, a higher BCS compared to the goats, possibly because in early lactation the goats lost more body reserves, as suggested by the evolution of BW and BCS in early lactation (Chapter 3) and by the energy balance, much higher in the ewes than in the goats (Table 8).

Milk fat concentration and milk protein concentration were higher in the ewes (fat: 4.83%; protein: 4.45%) than in the goats (fat: 3.80%; protein: 3.07%) ($P=0.0001$; $P<0.0001$; fat and protein; respectively) (Table 3). This is not surprising, due to the fact that always dairy ewes have higher concentration of fat and protein in the milk than dairy goats and cows (Park et al., 2007). However, while the fat and protein values of Saanen goats were close to those reported in the literature (Pulina et al., 2008), those of the Sarda ewes were much lower than the values usually observed in early lactation (Pulina et al., 2007), probably indicating a status of milk fat depression in this species, but not in goats, caused by the high-starch content of the diet. Abijaoudé et al. (2000) suggested a better ability of goats compared to sheep to avoid rumen acidosis, mainly due to the fact that starch-rich diets are eaten with many small meals, thus reducing the drops of rumen pH. This mechanism could explain the differences we observed. However, unfortunately feeding behavior was not monitored in this experiment. Due to the milk fat depression in sheep, the diet was slightly changed in mid-lactation, reducing the corn supplied at milking and introducing soyhulls, not used in early lactation, in the HS pellets with the goal of increasing the NDF concentration of the HS diet (Table 1).

Due to the much higher milk production, despite the lower milk fat and protein concentration, goats had a much higher fat and protein yield, and thus daily milk energy output, than the ewes.

Intake

The daily intake of DM and of all its chemical constituents was higher in the goats than in the ewes (Table 4). However, this difference was not evident when intake was

expressed in proportion of the BW of the animals, *i.e*. when the level of intake was considered. Indeed, the level of intake of DM was equal to 5.3% in goats and 5.0% in the ewes (Table 5). These values are in line with those typically observed in lactating goats and ewes (Pulina et al., 2013). The lack of species differences in terms of level of intake is somehow surprising, since the goats were much more productive and thus a higher level of intake of DM would have been expected. On this regards the literature gives contrasting indications regarding the feed intake of the two species. For example, Aregheore (1996), in a comparative study, observed that sheep had higher DMI than goats fed a crop residue based diet. Similarly, DMI and OMI was higher in Finn sheep (7 months old) than in Saanen and Alpine goats (12 months old) kept in metabolic cages and fed diets with different forage to concentrate ratios, whereas NDFI and ADFI were highest in goats (Ramanzin et al., 1997). In contrast, DMI was higher in goats than in ewes fed a tropical natural grass (*Hyparrhenia spp*.) (Gihad, 1976). The same authors suggested that goats tend to eat more poor quality tropical hay than ewes (Gihad, 1976). Similarly, the voluntary feed intake was higher in goats (56 g DM/kg $BW^{0.75}$) than in ewes (36 g DM/kg $BW^{0.75}$) fed a low-quality roughage (*Bromus catharticus*) (Domingue et al., 1991).

Differences in DMI depend and are positively affected by the quality of the diet. In a comparative study, Huston et al. (1988) observed that DMI increased in both species increasing the quality of the diet, thus going from a low quality forage (wheat straw; sheep: 29 ± 9 $g/kg^{0.75}$; goats 19 ± 10 $g/kg^{0.75}$) to a medium quality forage (sorghum hay; sheep: 39 ± 6 $g/kg^{0.75}$; goats 36 ± 12 $g/kg^{0.75}$) up to a high quality forage (oat hay; sheep: 64 ± 22 $g/kg^{0.75}$; goats 54 ± 24 $g/kg^{0.75}$), but was always higher in sheep than in goats. In another study, goats consumed more DM per kg $BW^{0.75}$ compared to ewes, when both species were fed five roughages offered alone or with concentrates (Antoniou and Hadjipanayiotou, 1985).

It appears that since the comparative studies refer to different breeds, feeding conditions, and production levels, it is difficult to make any generalization on this regard.

Digestibility

The in *vivo* digestibility coefficients (Table 6) did not differ between the two species, except for NFC apparent digestibility, higher in goats (98.46%) than in ewes (91.74%) ($P<0.0001$; Table 6). As a result of the similarity in digestibility and the higher intake of goats, the daily digested nutrient intake was higher in goats than in ewes (Table 7).

Probably, the lack of the differences between the two species in the digestibility coefficients is due to the quality of the diet offered. In fact, when the quality of the diet is low, goats seems to use the diet better than ewes, showing higher digestibility coefficients (Watson and Norton, 1982; Domingue et al., 1991; Aregheore, 1996). However, in this study the higher digestibility of goats was probably due to their lower DMI compared to sheep.

As suggested by Sales et al. (2011), goats seem to be able to better digest poor quality forages.

On the contrary, when the quality of the diet is intermediate or high, the two species do not differ (Huston et al., 1988; Isac et al., 1994; Alcaide et al., 2000; Sales et al., 2011). In fact, the digestibility coefficients did not differ in Granadina goats and Segureña wethers fed a medium quality forage (Isac et al., 1994) or a good quality diet (Alcaide et al., 2000). In addition, DM digestibility did not differ between Rambouillet ewes and Spanish goats fed a pellet containing 60% roughage, cottonseed hulls and alfalfa hay pellet (Houston et al., 1986). No interpecies differences in the apparent digestibility of nutrients were found when five roughages (barley, lucerne or sudax hay, barley straw and leaves and twigs of acacia) were offered alone or with concentrate (1:1 ratio) in a comparative study, using Chios ram and Damascus goats, except for the highest digestibility of DM of straw in goats (Antoniou and Hadjipanayiotou, 1985). However, as suggested by Hadjigeorgiou et al. (2000), many of these studies have been conducted by using tropical forages. The same authors studied the voluntary feed intake, digestibility and mean retention time in male sheep and male goats fed temperate forages and observed that, goast had a higher voluntary feed intake and lower digestibility compared to sheep, whereas the mean retention time did not differ between the two species. In addition, goats selected diets with high nutritive value and smaller particle size than sheep. The Authors concluded that the behavior of the two species was the same both for tropical than for temperate forages.

The lack of difference in digestibility can be interpreted considering that the level of intake of DM and of NDF was similar between the two species during the trial (Table 5). Indeed, Cannas and Van Soest (2000) and Cannas et al. (2003) suggested that at the same level of intake of NDF the rumen feed passage rate is similar in all ruminants, regardless their body size, and that dietary digestibility should be also similar.

Dietary energy value and energy balance of the animals

The energy content of the diets, estimated on the basis of the TDN measured in the digestibility trial, did not differ between the two species, even though there was a trend of greater energy values in goats than in ewes (P=0.10; Table 8). This, in combination to the higher DMI intake, explained the much higher MEI goats compared to ewes. Due to their higher BW and milk production, goats had also higher requirements for maintenance and lactation than ewes. Overall, the energy balance (energy intake - energy requirements for maintenance and lactation) of the goats was close to zero, while that the ewes was positive (0.06 vs. 0.75 Mcal of ME/d, P=0.069, for goats and sheep, respectively).

This difference in energy balance is in agreement with the patterns observed in early lactation on BW and BCS variations, which suggested higher BW loss and faster reserve recovery during early lactation in the ewes compared to the goats (Chapter 3). This could be due to the fact that milk production of the goats was very high in early lactation and peaked at 65 DIM, while that of sheep decreased continuously after weaning (Chapter 3). In addition, GH values of goats also peaked around 60 DIM and where much higher than those of the ewes (Chapter 3). Thus, it is possible to speculate that while goats were in a high catabolic status in early lactation, sheep were not, suggesting that the shift from negative to positive energy balance occurred much earlier in this species.

Rumen fermentations, microbial activity and N balance

Rumen and gross intestine pH were higher in goats than in ewes. This is an interesting finding, because confirms a better ability of goats than ewes to limit the decrease of the pH with high-starch diets, as previously suggested by Abijaoudé et la. (2000). Previous studies observed that rumen pH was lower in goats (6.73) than in ewes (6.90), but both

fed a low-quality roughage and low starch diets, with high absolute values of rumen pH in both species (Domingue et al., 1991).

The values were in generally low, due to the high-starch content of the diet and the very high intake of both species during the trial. In addition, the pH was measured in a sample taken from the whole rumen content, thus it included the liquid contained in the lower sac of the rumen, usually not sampled in rumen pH measurements and which typically has much lower pH than the upper part of the liquid fraction of the rumen.

The NH_3 concentration was much greater in goats (23.4 mg/dl) than in ewes (11.9 mg/dl) (P=0.005), whereas the VFA concentration did not differ between the two species (Table 9). The higher ammonia concentration in goats could indicate higher N recycling and a more intense microbial activity, since milk urea, representing wasted N excretion, did not differ between the two species. In another study rumen ammonia concentration was higher in goats (115 mg N/l) than in ewes (80 mg N/l), both fed a low-quality roughage, and this could have been a factor that caused the greater voluntary feed intake and fiber digestion in goats compared to sheep (Domingue et al., 1991).

As just said, microbial protein supply was markedly higher in goats than in ewes. However, the difference was probably associated to their higher DMI. Indeed, the microbial protein produced per kg of DM, CP and NDF eaten did not differ among species. The higher rumen ammonia in goats could also depend on their better ability to recycling N in the rumen, as suggested by Silanikove (2000). The lack of difference in VFA concentration between the two species could depend on different absorption rates by the rumen wall, possibly pH controlled.

In early lactation, no differences where observed in terms of total animal N balance, even though it was numerically greater in goats, suggesting that in both species there was an accumulation of body proteins. However, some caution should be taken in considering the N balance as an indicator of body protein accumulation or loss, as suggested by Spanghero and Kowalski (1997), who reported that N balances in ruminants are almost always positive, even when negative values are expected (e.g. early lactation), possibly for methodological reasons.

4.2 Mid-lactation

Milk production and body reserves

As expected, milk production, FCM and milk NE_L yield were higher in goats than ewes (Table 11). Milk fat and protein concentrations were higher in ewes than goats. Milk fat and protein concentration of the ewes was much higher than that observed in early lactation, being much closer to the values normally observed in this species (Pulina et al., 2007). In ewes, milk fat concentration was numerically higher with LS diets than with HS diets (7.15% vs. 6.72%), as earlier observed by Cannas et al. (1998), Zenou and Miron, (2005), and Cannas et al. (2013) in ewes fed diets in digestible fiber-rich sources.

The highest milk fat concentration observed in the LS diet was expected, considering the positive correlation between milk fat and fiber content of the diet as reported in the equation of Pulina and Rassu (1991) (fat=4.59 + 0.05 x NDF (r=0.48)). However, this effect seems to be due indirect. As suggested by Bencini and Pulina (1997), this relation is difficult to explain because high NDF diet reduces the digestibility of the diet and the feed intake, which in turn reduces milk production and increase milk fat.

Ewes compared to goats had much higher BCS, as already observed in the Chapters 2 and 3. This difference was much larger than that observed in early lactation, confirming the above mentioned findings, *i.e.* a much more intense body reserve accumulation, especially in the last experimental days, in ewes than goats.

Intake

In mid-lactation, the intake of DM and of all other chemical constituents was higher in goats than in ewes. In contrast to what observed in early lactation, the level of intake (% of BW) of DM and of all other chemical constituents was also significantly higher in goats than ewes. This is an interesting finding, since blood leptin, an anorexic hormone, was actually higher in goats than in ewes (Chapter 3) and its value did not increase during the lactation of the ewes, despite they very high accumulation of body reserves.

It should be mentioned, however, that during the digestibility trials carried out in mid-lactation all the animals, but especially the ewes, showed marked signs of discomfort and difficulties to adapt to the metabolic cages. This did not occur at all in early

lactation. It is possible that the high temperatures reached in the month of June (during which the trials were carried out and the maximum temperature ranged from 24 to 30°C and the relative humidity reached 100% for at least 50% of the time) during which the trials were carried out, caused a distress to the animals and that it was exacerbated but the high BCS of the ewes, and, to a lesser extent, of the goats (Chapter 2). Indeed, fat animals are more subjected to heat stress than lean animals (Brown-Brandl et al., 2006).

Regarding the effect of dietary treatments on intake, this was not significant except for NFC and starch, higher for the HS than for the LS group. Indeed, even though not significant, DMI was markedly higher in the HS than LS groups. When calculated as proportion of BW, DMI was almost significantly higher with the HS diet (P=0.08; Table 13). This higher intake could have been an effect of the numerically higher milk production of HS goats and of the heat stress in LS ewes, which notoriously is highest and limits DMI the most in animal fed high fiber diets, such as those using the LS diet.
It is well known that an increase in milk production is positively correlated with feed intake and metabolic heat production. E.g., and in high genetic merit cows milk production is markedly affected by the environmental temperatures (Kadzere et al., 2002). In fact, the reduction in feed intake and production is a homeostatic response to heat stress (Kadzere et al., 2002). When the diet is rich in concentrate and poor in forage, the heat stress is reduced because the ME derived from high concentrate diets is used more efficiently than the ME from high forage diets (Kadzere et al., 2002). Previous experiments observed a reduction in DMI, MEI, liveweight gain and N balance in sheep exposed to heat stress and fed a medium quality roughage diet (Dixon et al., 1999). The effect of the heat stress on the depression of feed intake was most severe in Awasi wethers fed diet containing barley hay and concentrate in a 75:25 ratio than the wethers that received the same diet with a 25:75 and 50:50 barley hay and concentrate ratio. In addition, ME intake was depressed at the maximum level of roughage (75%) whereas the nitrogen utilization was not affected (Bhattacharya and Hussain, 1974).
In addition, the heat stress decreased DMI and increased nutrients digestibility and changed hormonal profile, increasing insulin and IGF-I concentration and decreasing

GH and glucose concentration in goats exposed to high temperatures (Hirayama et al., 2004).

Digestibility

DM digestibility was not affected by the species, confirming the finding observed in early lactation. However, CP apparent digestibility and NDF true digestibility where higher in goats, while NFC and starch apparent digestibility were higher in the ewes (Table 14). It seems that goats could use in a better way the fiber, in opposition to what observed in ewes, which instead seemed to be more able to use starch. However, in early lactation, there were no species differences, except for NFC digestibility, which was higher in goats than ewes. Higher digestibility of nutrients in ewes than in goats was expected, due to the lower DMI observed in ewes compared to goats. In fact, differences in the level of intake can change the retention time of digesta (Sales et al., 2011), with low DMI associated with high rumen retention time and thus higher digestibility. However, this did not occur in this trial. No clear reasons can be given for this contrasting results, except that the highest CP digestibility and microbial N supply of the goats might have favored the NDF rumen degradation in mid lactation.

The differences in the capacity to digest the nutrients between the two species is still under debate, as suggested by the contrasting results observed in the literature.

Ramanzin et al. (1997) comparing the two species observed that ewes had a typical behavior of specialized grazers, with lower selectivity, higher feed intake and higher fiber digestibility than goats. Sales et al. (2011), in a review of the literature concerning the differences in total tract nutrient digestibility between ewes and goats, observed that in all of the experiment cited, DM, OM, NDF and ADF digestibility was higher in goats than in ewes ($P<0.05$) when fed all forage diets were used, whereas did not differ when the concentrate was included in the diet. Gihad et al. (1980) suggested that goats can use and digest better the fiber than ewes and use better poor quality roughage.

All but ash in vivo digestibility coefficients were significantly affected by the dietary treatments. The values were higher for the HS diet than for the LS diet for all chemical constituents, except the fiber fractions (NDF and ADF), more digestible with the LS than the HS diets; in addition, TDN was highest with the HS diet (Table 14). The high NDF digestibility of LS diets is certainly due to their high content of soyhulls, which

have a very digestible fiber (Van Soest, 1994). These results are in accordance with an experiment conducted in mid-lactating ewes, with high NFC diet that had higher DM, OM, CP apparent digestibility, and TDN and lower NDF true digestibility compared to low NFC diets (Cannas et al., 2013). In a study conducted in mid lactating goats, high-starch diets had higher DM, OM, CP apparent digestibility compared to low-starch diets. In this study, however, NDF digestibility was not affected by the treatments (Ibáñez et al., 2015). In a comparative study using simultaneously the two species, OM and CP apparent digestibility decreased increasing the forage to concentrate ratio (from 10:90 to 90: 10) in opposition to what observed for NDF and ADF apparent digestibility, which instead increased (Ramanzin et al., 1997).

Dietary energy value and energy balance of the animals

The dietary DE, ME, and NE_L concentrations did not differ between the two species but were markedly higher for the HS than for the LS diet (Table 16). In accordance to what observed in early lactation, MEI was higher in goats than in ewes and was numerically greater (P=0.07) for the HS than for the LS diets. In addition, energy requirements for maintenance, lactation and their sum were highest in goats (P<0.0001), as expected considering their high BW and milk production, while there were not differences between the HS and LS groups. Despite the higher requirements of goats, their energy balance was not different from that of the ewes, since goats had also a higher MEI. This was probably caused by conditions of heat stress, which limited the intake in both species, but likely more in sheep than goats, due to the high BCS of the former. Indeed, the energy balance of sheep was negative for both dietary treatments, despite their low milk production, suggesting a negative effect of the environmental temperature and humidity and of the confinement in the metabolic cages on their energy intake.

The effect of the diets was significant, with the values for the LS diets much lower and negative in both species than those for the HS diets. Indeed, there was a large variability within the experimental groups, caused by a large variability in milk yield. Overall, it appears that the LS diet, being richer in fiber, induced, during the time in the metabolic cage, a stronger thermal discomfort, and thus a decrease in MEI stronger than in the case of the groups fed HS diets.

In accordance to our results, in mid lactating ewes dietary ME and NEL concentrations were higher for high than low NFC diets, whereas MEI and total energy requirements did not differ between the two dietary groups (Cannas et al., 2013). In the same study, the EB estimated in mid lactating ewes was markedly positive and higher than the EB observed in our experiment. In the study of Cannas et al. (2013), the diets were pelleted and this probably reduced possible negative effects associated to the observed heat stress. In addition, the EB it was numerically higher with high than low NFC diets (1.895 vs. 1.501 ± 0.227; respectively; P<0.099) (Cannas et al., 2013), similarly to what found in our experiment.

Rumen fermentations, microbial activity and N balance

Differently than what observed in early lactation, rumen pH did not differ between goats and ewes. However, the cecum and colon pH was higher in goats than in ewes. The lack of species difference at rumen level was possibly caused by the lower intake. Especially in ewes, observed in mid-lactation compared to early lactation. Indeed, rumen pH values were generally higher, even for the HS diets. It is also possible that the slight change in composition between the HS diets used in early lactation compared to mid-lactation (Tables 1 and 2), with an increase in soyhulls and NDF, limited the pH drop.

In contrast, the dietary treatments had a marked effect on pH at rumen level, with HS diets having lower values, but not in the intestine. While the lower pH at rumen level for HS diets was expected, the lack of difference in the gross intestine is not easily explained. However, it should be noted that for both diets the values where high and in line with the optimal pH values reported for cecum and colon (Van Soest, 1994).

According to what observed in early lactation, NH_3 concentration was greater in goats than in ewes (P=0.004). This can be explained with the same motivation given for early lactation, *i.e.* a more intense N recycling in goats than in ewes. Indeed, microbial protein flow was higher in goats than in ewes, even though when microbial protein flow was expressed in proportion of DM, CP and NDF eaten, there were no species differences. The dietary treatments did not affect ammonia concentration and microbial protein flow, suggesting that microbial activity and dietary protein degradation was not affected by the diets. Possibly, even the LS diet provided sufficient energy at rumen

level to maximize microbial activities. This result is in contrast to the higher microbial flow of diets with high digestible fiber content compared to diet with high-starch content observed by Cannas et al. (2004). In that experiment, however, the daily DMI, and thus likely the feed rumen passage rate was much higher than in our experiment. This might have increased the efficiency of microbial synthesis, as suggested by Van Soest (1994), especially in high fiber diets, which usually have higher rumen passage rate.

Regarding the VFA, the molar concentration of propionic and iso-butyrric acid was highest in sheep (Table 17). In terms of molar proportions, the acetic acid was higher in goats than in ewes. No clear reasons can be given to explain these differences in VFA concentrations and proportions. However, it should be considered that the differences observed, even when significant were small and biologically not very relevant.

The acetic acid molar proportion was higher in LS diets and that of butyric acid was higher in HS diets. This is in line with the well-known stimulatory effect of high fiber diets on acetic acid prevalence.

According to what observed in early lactation, no differences where observed in terms of protein mobilization between the species, as suggested by the fact that the N balance that did not differ between ewes and goats. The N balance was affected by the diet, being more positive in both species with the HS diets.

5. CONCLUSIONS

In conclusion, this study highlighted some nutritional differences between goats and ewes both in early and mid-lactation.

In early lactation, goats had much higher DMI, due to their higher milk production, higher rumen pH, ammonia concentration and microbial protein flow than the ewes, while digestibility was not affected by species. When the microbial flow was calculated in proportion of the DMI, there were not species differences. In mid-lactation the pattern was somehow similar, even though rumen pH did not differ and goats showed a better ability to digest the fiber of the diet, while the ewes had a better digestibility of starch.

Overall, these results do not suggest any specific nutritional effect in terms of digestibility and rumen fermentations that could explain the productive differences observed in Chapter 2 comparing the two species under two different dietary starch concentrations.

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7. TABLES

Table 1. Ingredient and chemical composition of the high-starch (HS) and low-starch (LS) diets supplied during the during the digestibility trials.

Period	**Early lactation**	**Mid lactation**	
Groups/Diet	**HS**	**HS**	**LS**
Ingredients (% as fed)			
Pellet (high-starch or low-starch)	65.0	67.0	67.0
Dehydrated chopped alfalfa	32.0	29.0	29.0
Whole corn grain	*	*	*
Mature ryegrass hay	3.0	4.0	4.0
TOTAL	*100.00*	*100.0*	*100.0*
Chemical composition[a]			
DM (% as fed)	88.6	89.6	89.1
CP (% DM)	16.2	15.5	15.6
Ash (% DM)	10.7	11.0	11.2
Ether extract (% DM)	2.3	1.4	1.4
NDF (% DM)	35.4	36.7	48.8
ADF (% DM)	21.5	25.6	35.5
ADL (% DM)	3.6	4.7	5.1
NFC (% DM)[b]	35.5	35.4	23.0
Starch (% DM)	20.4	20.0	7.8

[a] The chemical composition does not include the corn grains supplied at milking; [b] NFC: 100 – CP – ash – NDF - ether extract; * additional supply of whole corn grains, 200 g/d in early lactation and 100 g/d in mid lactation, with the following chemical composition: DM 86.5%, as fed; on a DM basis: CP 8.0%, ash 1.43%, fat 2.1%, NDF 16.7%, ADF 4.7%, ADL 0.9%, NFC 71.8%, starch 69.6%

Table 2. Ingredients and chemical composition of the high-starch and low-starch pellets supplied during the digestibility trials.

Period	**Early lactation**	**Mid-lactation**	
Groups/Pellet	**High-starch**	**High-starch**	**Low-starch**
Ingredients (% as fed)			
Dehydrated alfalfa	30.5	30.0	30.0
Corn meal	26.9	21.1	3.0
Barley meal	13.4	13.4	0.0
Wheat bran	10.1	10.1	5.0
Soybean hulls	-	9.0	43.2
Soybean meal 44	7.9	5.0	7.4
Sugarcane molasses	4.6	4.6	4.6
Sodium bicarbonate	4.3	3.0	3.0
Bentonite	2.0	2.0	2.0
Magnesium oxide	-	1.5	1.5
Minerals and vitamins	0.3	0.3	0.3
Appetizer	0.03	0.03	0.03
TOTAL	100.0	100.0	100.0
Chemical composition			
DM (% as fed)	88.6	90.0	89.3
CP (% DM)	15.2	14.1	14.2
Ash (% DM)	10.7	11.3	11.6
Ether extract (% DM)	2.5	1.4	1.4
NDF (% DM)	27.4	30.7	48.8
ADF (% DM)	14.6	19.9	34.7
ADL (% DM)	1.5	2.9	3.6
NFC (% DM)[a]	44.2	42.6	24.0
Starch (% DM)	30.0	28.1	10.0

[a] NFC: 100 – CP – ash – NDF - ether extract

Table 3. Body weight (BW), body condition score (BCS) and milk production in goats and sheep fed high-starch (HS) diet during the digestibility trial in early lactation.

	Species		SEM[a]	*P* level
	Goats	Sheep		S[b]
Milk yield (kg/d)	4.31	2.23	0.43	0.0001
FCM (kg/d)[d]	4.42	1.86	0.44	<0.0001
NE_L (Mcal NE/d)[e]	3.11	1.91	0.33	0.002
Fat (%)	3.80	4.83	0.21	0.0001
Fat (g/d)	163.04	107.05	19.64	0.011
Protein (%)	3.07	4.45	0.16	<0.0001
Protein (g/d)	132.37	98.10	14.61	0.031
Lactose (%)	4.71	4.85	0.10	N.S.[c]
Lactose (g/d)	201.61	108.59	19.34	0.0001
Urea (mg/dl)	43.02	46.41	2.73	N.S.
SCC (log)[f]	2.59	2.42	0.27	N.S.
BW (kg)	57.81	47.41	2.99	0.003
BCS (scale 0-5)	2.67	2.79	0.08	N.S.

[a] Standard error of the mean; [b] effect of species; [c] $P>0.05$; [d] fat-corrected milk yield; [e] net energy for lactation; [f] somatic cell cont

Table 4. Intake (expressed in kg/d) of the goats and sheep fed high-starch (HS) diet during the digestibility trial in early lactation.

	Species		SEM[a]	*P* level
	Goats	Sheep		S[b]
Intake (as fed)	3.48	2.64	0.40	0.049
DM intake	3.09	2.35	0.36	0.051
Ash intake	0.31	0.23	0.04	0.052
OM intake	2.78	2.12	0.32	0.052
FAT intake	0.07	0.05	0.008	0.063
CP intake	0.49	0.36	0.06	0.045
NDF intake	1.03	0.78	0.12	0.046
ADF intake	0.60	0.46	0.07	0.042
ADL intake	0.10	0.07	0.01	0.018
NFC intake	1.19	0.92	0.13	0.063
Starch intake	0.74	0.58	0.08	0.078

[a] Standard error of the mean; [b] effect of species

Table 5. Level of intake (expressed in % of body weight) of the goats and sheep fed high-starch (HS) diet during the digestibility trial in early lactation.

	Species		SEM[a]	*P* level
	Goats	Sheep		S[b]
DM intake	5.32	5.00	0.59	N.S.[c]
Ash intake	0.54	0.50	0.06	N.S.
OM intake	4.78	4.50	0.53	N.S.
FAT intake	0.11	0.11	0.01	N.S.
CP intake	0.84	0.77	0.10	N.S.
NDF intake	1.79	1.66	0.20	N.S.
ADF intake	1.04	0.97	0.11	N.S.
ADL intake	0.17	0.16	0.02	N.S.
NFC intake	2.04	1.96	0.22	N.S.
Starch intake	1.27	1.24	0.13	N.S.

[a] Standard error of the mean; [b] effect of species; [c] P>0.05

Table 6. In *vivo* digestibility coefficients (expressed in %, on DM) of the high-starch (HS) diet supplied the digestibility trials to the goats and sheep in early lactation.

	Species		**SEM**[a]	***P* level**
	Goats	**Sheep**		**S**[b]
DM apparent digestibility	69.15	69.40	1.16	N.S.[c]
ASH apparent digestibility	52.20	50.52	2.24	N.S.
OM apparent digestibility	71.05	71.50	1.06	N.S.
FAT apparent digestibility	66.34	68.49	1.29	N.S.
CP apparent digestibility	70.64	70.86	1.17	N.S.
NDF true digestibility	46.75	48.04	2.09	N.S.
ADF true digestibility	38.71	39.49	2.46	N.S.
NFC apparent digestibility	98.46	91.74	0.33	<0.0001
Starch apparent digestibility	97.52	97.90	0.34	N.S.
TDN[d]	67.88	66.20	0.99	0.10

[a] Standard error of the mean; [b] effect of species; [c] P>0.10; [d] total digestible nutrients, calculated applying the in *vivo* digestibility coefficient obtained to equation n. 25.3 of Van Soest (1994)

Table 7. Daily digested nutrients intake (expressed in kg/d) of the goats and sheep fed high-starch (HS) diet during the digestibility trial in early lactation.

	Species		SEM[a]	*P* level
	Goats	Sheep		S[b]
Digested DM intake	2.13	1.62	0.23	0.042
Digested ASH intake	0.16	0.12	0.02	0.016
Digested OM intake	1.97	1.50	0.21	0.045
Digested FAT intake	0.04	0.03	0.006	0.054
Digested CP intake	0.34	0.26	0.04	0.034
Digested NDF intake	0.48	0.37	0.05	0.043
Digested ADF intake	0.23	0.18	0.03	0.062
Digested NFC intake	1.17	0.84	0.13	0.021
Digested starch intake	0.72	0.57	0.08	0.078

[a] Standard error of the mean; [b] effect of species

Table 8. Energy value of the high-starch (HS) diet supplied to the goats and sheep in early lactation during the digestibility trial and their energy requirements and energy balance.

	Species		SEM[a]	*P* level
	Goats	**Sheep**		**S**[b]
Energy value of the diet				
DE (Mcal/kg SS)[c]	2.99	2.92	0.04	0.10
ME (Mcal/kg SS)[d]	2.45	2.39	0.03	0.09
NE_L (Mcal/kg SS)[e]	1.58	1.54	0.02	0.10
MEI (Mcal ME/d)[f]	7.56	5.59	0.83	0.03
Energy requirements				
Maintenance (ME_M; Mcal ME_M/d)[g]	2.67	1.87	0.13	<0.0001
Lactation (ME_L; Mcal ME_L/d)[h]	4.84	2.97	0.51	0.0019
Total Energy requirements (Mcal ME/d)[i]	7.50	4.84	0.62	0.0005
Energy Balance (EB; Mcal ME/d)[l]	0.06	0.75	0.35	0.069

[a] Standard error of the mean; [b] effect of species; [c] digestible energy; [d] metabolizable energy; [e] net energy for lactation; [f] metabolizable energy ingested; [g] metabolizable energy for maintenance; [h] metabolizable energy for lactation; [i] $ME_M + ME_L$; [l] MEI - ME_M –ME_L

Table 9. Rumen and lower tract pH, volatile fatty acid and ammonia (NH_3) in ruminal liquid collected at the end of the digestibility trial from goats and sheep fed high-starch (HS) diet in early lactation.

	Species		SEM[a]	*P* level
	Goats	Sheep		S[b]
Ruminal pH	5.86	5.70	0.07	0.052
Cecum and colon pH	7.04	6.71	0.16	0.058
NH_3 (mg/dl)	23.38	11.89	3.46	0.005
Acetic acid (mMol/L)	92.20	99.53	9.48	N.S.[c]
Propionic acid (mMol/L)	26.16	31.74	4.31	N.S.
Butyric acid (mMol/L)	1.41	0.93	0.48	N.S.
Iso-butyric acid (mMol/L)	26.04	25.98	2.94	N.S.
Iso-valerianic acid (mMol/L)	1.07	0.65	0.35	N.S.
Valerianic acid (mMol/L)	2.33	2.09	0.22	N.S.
Acetic acid (%)*	63.74	63.45	1.52	N.S.
Propionic acid (%)*	17.82	19.95	1.26	N.S.
Butyric acid (%)*	18.43	16.60	2.03	N.S.

[a] Standard error of the mean; [b] effect of species; [c] not significant $P>0.10$; * Percent of the sum of acetic, propionic and butyric acid

Table 10. Microbial protein flow and N balance of the goats and sheep fed high-starch (HS) diet during the digestibility trial in early lactation.

	Species		SEM[a]	*P* level
	Goats	**Sheep**		**S**[b]
Microbial Protein (g/d)	130.64	90.72	14.62	0.0137
Microbial Protein/kg DM	40.35	39.86	2.92	N.S.[c]
Microbial Protein/kg CP	268.78	255.10	19.12	N.S.
Microbial Protein/kg NDF	123.55	117.38	8.82	N.S.
N balance (g/d)	11.49	9.26	2.84	N.S.

[a] Standard error of the mean; [b] effect of species; [c] $P>0.05$

Table 11. Milk production, body weight (BW) and body condition score (BCS) of the goats and sheep fed high-starch (HS) and low-starch (LS) diets during the digestibility trial in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
Milk (kg/d)	2.42	2.13	0.96	0.85	0.17	<0.0001	N.S.[h]	0.0004	N.S.	0.024	N.S.
FCM (kg/d)[i]	2.35	2.06	0.98	0.87	0.17	<0.0001	N.S.	0.0009	N.S.	0.004	N.S.
NE_L (Mcal/d)[l]	1.65	1.46	1.02	0.89	0.13	<0.0001	N.S.	0.0017	N.S.	0.009	N.S.
Fat (%)	3.11	3.11	6.72	7.15	0.20	<0.0001	N.S.	0.009	N.S.	<0.0001	N.S.
Fat (g/d)	78.48	68.58	65.06	57.11	7.97	N.S.	N.S.	0.0043	N.S.	0.004	N.S.
Protein (%)	3.08	3.13	5.14	5.03	0.11	<0.0001	N.S.	0.012	N.S.	N.S.	N.S.
Protein (g/d)	74.04	66.90	48.75	50.00	5.64	<0.0001	N.S.	0.0017	N.S.	0.049	N.S.
Lactose (%)	4.38	4.33	4.43	4.19	0.10	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Lactose (g/d)	106.41	91.88	42.86	35.03	7.44	<0.0001	N.S.	0.0013	N.S.	0.03	N.S.
Urea (mg/dl)	34.73	41.24	26.67	26.04	1.38	<0.0001	0.041	N.S.	0.014	0.013	N.S.
SCC (log)[m]	2.93	2.94	2.82	2.99	0.14	N.S.	N.S.	N.S.	N.S.	0.064	N.S.

Table 11. (continued).

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
BW (kg)	59.73	63.59	54.57	57.16	2.19	0.013	N.S.	N.S.	N.S.	N.S.	N.S.
BCS (scale 0-5)	2.82	2.76	3.19	3.30	0.08	<0.0001	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$; [i] fat-corrected milk yield; [l] net energy for lactation; [m] somatic cell cont

Table 12. Intake (expressed in kg/d) of the goats and sheep fed high-starch (HS) and low-starch (LS) diets during the digestibility trial in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
Intake (as fed)	2.46	2.21	1.60	1.39	0.22	0.0005	N.S.[h]	N.S.	N.S.	N.S.	N.S.
DM intake	2.23	1.98	1.44	1.23	0.20	0.0005	N.S.	N.S.	N.S.	N.S.	N.S.
Ash intake	0.23	0.21	0.15	0.13	0.02	0.0004	N.S.	N.S.	N.S.	N.S.	N.S.
OM intake	2.00	1.77	1.29	1.10	0.18	0.0004	N.S.	N.S.	N.S.	N.S.	N.S.
FAT intake	0.03	0.03	0.02	0.02	0.003	0.0011	N.S.	N.S.	N.S.	N.S.	N.S.
CP intake	0.33	0.30	0.21	0.18	0.03	0.0002	N.S.	N.S.	N.S.	N.S.	N.S.
NDF intake	0.80	0.94	0.51	0.58	0.09	0.0012	N.S.	0.09	N.S.	N.S.	N.S.
ADF intake	0.56	0.69	0.35	0.41	0.06	0.0007	N.S.	N.S.	N.S.	N.S.	N.S.
ADL intake	0.10	0.10	0.06	0.06	0.10	0.0001	N.S.	N.S.	N.S.	N.S.	N.S.
NFC intake	0.83	0.50	0.55	0.33	0.06	0.0003	<0.0001	N.S.	N.S.	N.S.	N.S.
Starch intake	0.49	0.21	0.33	0.14	0.03	0.0007	<0.0001	0.02	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$

Table 13. Level of intake (expressed in % of body weight) of the goats and sheep fed high-starch (HS) and low-starch (LS) diets during the digestibility trial in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
DM intake	3.74	3.12	2.68	2.13	0.33	0.004	0.08	N.S.[h]	N.S.	N.S.	N.S.
Ash intake	0.39	0.33	0.27	0.22	0.03	0.0036	N.S.	N.S.	N.S.	N.S.	N.S.
OM intake	3.34	2.79	2.40	1.91	0.29	0.0037	0.08	N.S.	N.S.	N.S.	N.S.
FAT intake	0.053	0.045	0.038	0.030	0.005	0.0035	0.09	N.S.	N.S.	N.S.	N.S.
CP intake	0.56	0.47	0.38	0.31	0.05	0.0018	0.10	N.S.	N.S.	N.S.	N.S.
NDF intake	1.33	1.48	0.96	0.99	0.15	0.0057	N.S.	N.S.	N.S.	N.S.	N.S.
ADF intake	0.93	1.09	0.65	0.71	0.10	0.0034	N.S.	N.S.	N.S.	N.S.	N.S.
ADL intake	0.17	0.16	0.12	0.10	0.01	0.0009	N.S.	N.S.	N.S.	N.S.	N.S.
NFC intake	1.40	0.79	1.02	0.57	0.10	0.0047	<0.0001	N.S.	N.S.	N.S.	N.S.
Starch intake	0.81	0.33	0.61	0.24	0.05	0.012	<0.0001	0.049	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$

Table 14. In *vivo* digestibility coefficients (expressed in %) of the high-starch (HS) and low-starch (LS) diets supplied during the digestibility trial to ewes and goats in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
DM apparent digestibility	68.50	64.32	67.97	64.25	0.73	N.S.[h]	<0.0001	N.S.	N.S.	N.S.	0.01
ASH apparent digestibility	48.03	43.14	43.19	42.10	1.82	N.S.	N.S.	0.08	N.S.	N.S.	N.S.
OM apparent digestibility	70.84	66.74	70.72	66.71	0.69	N.S.	<0.0001	N.S.	N.S.	N.S.	0.007
FAT apparent digestibility	54.64	48.67	52.48	47.03	1.07	0.09	<0.0001	<0.0001	N.S.	0.004	0.009
CP apparent digestibility	71.11	66.71	68.10	64.67	0.74	0.002	<0.0001	0.024	N.S.	0.038	0.001
NDF true digestibility	68.35	72.67	52.10	58.77	0.99	<0.0001	<0.0001	<0.0001	N.S.	<0.0001	0.052
ADF true digestibility	49.21	57.09	50.59	56.28	1.20	N.S.	<0.0001	0.004	N.S.	0.024	0.09
NFC apparent digestibility	74.64	54.21	88.44	82.21	2.38	<0.0001	<0.0001	<0.0001	0.005	<0.0001	0.002
Starch apparent digestibility	96.80	91.61	98.37	96.21	1.03	0.006	0.001	N.S.	N.S.	N.S.	N.S.
TDN[i]	64.35	60.58	64.39	60.59	0.62	N.S.	<0.0001	0.10	N.S.	N.S.	0.004

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$; [i] total digestible nutrients, calculated applying the in *vivo* digestibility coefficient obtained to equation n. 25.3 of Van Soest (1994)

Table 15. Daily digested nutrients intake (expressed in kg/d) of the goats and sheep fed high-starch (HS) and low-starch (LS) diets during the digestibility trial in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
Digested DM intake	1.53	1.26	0.97	0.79	0.12	0.0002	0.07	N.S.[h]	N.S.	N.S.	N.S.
Digested ASH intake	0.11	0.09	0.06	0.05	0.009	<0.0001	0.08	N.S.	N.S.	N.S.	N.S.
Digested OM intake	1.42	1.17	0.90	0.73	0.11	0.0002	0.07	N.S.	N.S.	N.S.	N.S.
Digested FAT intake	0.018	0.014	0.01	0.009	0.002	0.008	0.057	N.S.	N.S.	N.S.	N.S.
Digested CP intake	0.24	0.20	0.14	0.12	0.02	<0.0001	N.S.	N.S.	N.S.	N.S.	N.S.
Digested NDF intake	0.56	0.71	0.27	0.34	0.07	<0.0001	N.S.	0.003	N.S.	0.014	N.S.
Digested ADF intake	0.27	0.39	0.17	0.23	0.03	0.0004	0.012	N.S.	N.S.	N.S.	N.S.
Digested NFC intake	0.61	0.25	0.48	0.27	0.04	N.S.	<0.0001	0.020	0.07	0.003	N.S.
Digested starch intake	0.47	0.19	0.32	0.14	0.03	0.001	<0.0001	0.012	0.088	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$

Table 16. Energy value of the high-starch (HS) and low-starch (LS) diets supplied to the goats and sheep in mid-lactation during the digestibility trial and their energy requirements and energy balance.

	Goats		Sheep		SEM[a]	*P* level					
	HS	**LS**	**HS**	**LS**		**S**[b]	**D**[c]	**P**[d]	**SxD**[e]	**SxP**[f]	**DxP**[g]
Energy value of the diet											
DE (Mcal/kg SS)[i]	2.84	2.67	2.84	2.67	0.03	N.S.[h]	<0.0001	0.087	N.S.	N.S.	0.005
ME (Mcal/kg SS)[l]	2.33	2.19	2.33	2.19	0.02	N.S.	<0.0001	0.10	N.S.	N.S.	0.004
NEL(Mcal/kg SS)[m]	1.50	1.41	1.50	1.41	0.01	N.S.	<0.0001	0.098	N.S.	N.S.	0.004
MEI (Mcal ME/d)[n]	5.21	4.29	3.33	2.68	0.41	0.0002	0.07	N.S.	N.S.	N.S.	N.S.
Energy requirements											
Maintenance ($ME_{M;}$ Mcal ME_M/d)[o]	2.50	2.52	1.82	1.82	0.07	<0.0001	N.S.	N.S.	N.S.	N.S.	N.S.
Lactation (ME_L; Mcal ME_L/d)[p]	2.57	2.27	1.58	1.38	0.21	<0.0001	N.S.	0.002	N.S.	0.01	N.S.
Total Energy requirements (Mcal ME/d)[q]	5.08	4.79	3.40	3.20	0.26	<0.0001	N.S.	0.003	N.S.	0.03	N.S.
Energy Balance (EB; Mcal ME/d)[r]	0.13	-0.50	-0.07	-0.52	0.22	N.S.	0.02	N.S.	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$; [i] digestible energy; [l] metabolizable energy; [m] net energy for lactation; [n] metabolizable energy ingested; [o] metabolizable energy for maintenance; [p] metabolizable energy for lactation; [q] $ME_M + ME_L$; [r] energy balance (MEI - $ME_M - ME_L$)

Table 17. Rumen and lower tract pH, volatile fatty acid and ammonia (NH_3) in ruminal liquid collected at the end of the digestibility trial from goats and sheep fed high-starch (HS) and low-starch (LS) diets in mid-lactation.

	Goats		Sheep		SEM[a]	*P* level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
Ruminal pH	5.97	6.21	6.08	6.16	0.06	N.S.[h]	0.018	N.S.	N.S.	N.S.	N.S.
Cecum and colon pH	7.05	7.20	6.95	6.86	0.06	0.0015	N.S.	N.S.	0.057	0.015	N.S.
NH_3 (mg/dl)	25.66	26.32	17.71	20.08	2.21	0.004	N.S.	0.103	N.S.	N.S.	N.S.
Acetic acid (mMol/L)	104.23	88.01	99.81	102.45	5.51	N.S.	N.S.	0.021	0.10	N.S.	N.S.
Propionic acid (mMol/L)	24.15	20.88	25.58	27.66	2.23	0.078	N.S.	0.033	N.S.	N.S.	N.S.
Butyric acid (mMol/L)	24.76	15.21	22.60	19.86	1.24	N.S.	<0.0001	<0.0001	0.012	N.S.	0.002
Iso-butyric acid (mMol/L)	1.10	0.93	1.14	1.19	0.06	0.018	N.S.	0.0006	0.08	N.S.	0.096
Iso-valerianic acid (mMol/L)	1.05	0.73	0.91	0.87	0.06	N.S.	0.007	<0.0001	0.032	N.S.	0.0008
Valerianic acid (mMol/L)	3.77	2.75	3.30	4.95	0.44	0.061	N.S.	0.0001	0.006	N.S.	N.S.
Acetic acid (%)*	66.92	70.23	65.95	67.33	0.63	0.005	0.001	0.002	N.S.	N.S.	N.S.
Propionic acid (%)*	15.47	16.50	16.78	18.06	0.89	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Butyric acid (%)*	20.67	14.37	21.01	17.23	1.38	N.S.	0.001	0.023	N.S.	0.096	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.10$;
* Percent of the sum of acetic, propionic and butyric acid

Table 18. Microbial protein flow and N balance of the goats and sheep fed high-starch (HS) and low-starch (LS) diet during the digestibility trial in mid-lactation.

	Goats		Sheep		SEM[a]	P level					
	HS	LS	HS	LS		S[b]	D[c]	P[d]	SxD[e]	SxP[f]	DxP[g]
Microbial Protein (g/d)	94.71	89.69	57.82	55.72	7.19	<0.0001	N.S.[h]	0.037	N.S.	N.S.	N.S.
Microbial Protein/ kg DMI	43.40	47.34	42.23	48.35	3.34	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Microbial Protein/ kg CPI	284.92	311.35	280.21	317.83	22.24	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Microbial Protein/ kg NDFI	120.44	99.72	118.32	102.22	8.00	N.S.	0.028	N.S.	N.S.	N.S.	N.S.
N balance (g/d)	8.02	4.78	6.80	2.61	1.72	N.S.	0.039	N.S.	N.S.	N.S.	N.S.

[a] Standard error of the mean; [b] effect of species; [c] effect of diet; [d] effect of period; [e] species x diet interaction; [f] species x period interaction; [g] diet x period interaction; [h] $P>0.05$

CHAPTER 5

1. GENERAL DISCUSSION AND CONCLUSIONS

This work was developed to evaluate the effect of dietary carbohydrates, in particular starch vs. digestible fiber, in mid lactating ewes and goats. The information available in the literature (Chapter 1) evidenced that in mid-lactation the two species responded differently to the carbohydrates of the diet, even though none of the studies actually compared the two species in the same conditions. Lactating goats had a better milking persistency when fed high starch diets in mid-lactation, while in the same stage lactating ewes responded negatively to high starch diets, losing milk and increasing their body reserves. In contrast, the utilization of digestible fiber rich diets in this stage improved the lactation persistency. Thus, the aim of this work was to compare ewes and goats simultaneously, to evaluate their response to the type of carbohydrates of the diet and to understand which mechanisms were behind the different responses.

The different behavior of mid-lactating ewes and goats in relation to the carbohydrates of the diet was confirmed in the experiment carried out (Chapter 2), in which the effect of high-starch (HS) and low-starch digestible fiber-rich diets (LS) was tested in mid lactating ewes and goats studied simultaneously with the same environmental and management conditions. The results showed that, in mid-lactation when milk production decreases and the animals tend to accumulate body reserves, the effect of dietary carbohydrates was different between ewes and goats. In particular, in goats the HS diet favored milk production persistency, according to what observed in previous studies (Rapetti et al., 2005; Cannas et al., 2007; Ibáñez et al., 2015). In ewes, the HS diet stimulated body fat accumulation, penalizing milk production, whereas the LS diet limited the natural tendency to accumulate body fat in mid-lactation and favored milk production persistency, in accordance with previous studies (Cavani et al., 1990; Cannas et al., 2002, 2004; Bovera et al., 2004; Zenou and Miron, 2005; Cannas et al., 2013).

1.1 Possible explanations to explain the differences observed

Various mechanisms can be considered to explain the results observed in the experiment. For this reason, the research assessed the metabolic and hormonal status of

the animals, measured their diet digestibility, studied their ruminal and lower tract fermentations, and assessed their N balance and microbial protein production.

Hormonal and metabolic status of the animals

The mechanism that control this difference can be explained with the results obtained studying the evolution of the hormonal and metabolic profile during the lactation in the two species (Chapter 3), monitored from early to mid-lactation in the same feeding and management conditions. The growth hormone (GH) to insulin ratio observed, which is a positive indicator of the galactopoietic activity and of the energy partitioning towards mammary gland rather than body reserves (Welt and Wilhelmi, 1950; Luft and Guillemin, 1974; Hart, 1983; Davis and Collier, 1985), was always higher in the goats than in the ewes. This explained the positive effect of HS diet on milk production observed in goats. In contrast, the low GH to insulin ratio, an indicator of body fatness and energy partitioning towards adipose tissue (Rosi et al., 2009), of the ewes explained the negative effect of HS diet on their milk production and their higher body reserves accumulation. However, the dietary treatments (HS vs. LS) did not change the hormonal and metabolic status of the animals used in this experiment, in contrast to what observed in some publications, which reported that insulin hormone increases with starchy diets both in goats (Magistrelli et al., 2005) and in ewes (Cannas et al., 2004), whereas GH in some cases was increased with energy poor diets both in goats (Wang et al., 2016) and in ewes (Cannas et al., 2004). However, the literature on the effect of dietary carbohydrates on GH and insulin is not always consistent and in many instances no effects were observed (Breier, 1999).

Digestibility of the diets

The information obtained in the digestibility trials (Chapter 4), carried out simultaneously the two species both in early and mid-lactation, allowed an accurate estimation of the digestibility of the nutrients of the diets and of estimation of the energy balance of the animals. The species differences observed were small and explained mostly as effect of the different DMI and body size of the animals. They could no suggest any specific nutritional mechanism that would explain the productive

differences between the two species. The digestibility trials showed, as expected, that the HS diet was more digestible than the LS diet.

Rumen and cecum-colon pH, rumen ammonia volatile fatty acids, microbial protein supply, and N balance of the animals

The measurements carried out on the rumen and cecum-colon pH, rumen ammonia volatile fatty acids, microbial protein supply, and N balance of the animals gave important information about the feeding behavior of the two species. In goats, the higher rumen and cecum-colon pH observed indicates a better ability of goats to use starchy diets compare to ewes. Abijaoudé et al. (2000) suggested a better ability of goats compared to sheep to avoid rumen acidosis, mainly due to the fact that starch-rich diets are eaten with many small meals, thus reducing the drops of rumen pH. However, feeding behavior was not monitored in this experiment and rumen pH differed between the two species only in early lactation and not in mid-lactation. The lack of the differences in the acetate to propionate concentration did not help in explaining how the dietary carbohydrates affected milk production and body reserves accumulation. It should be noticed that the concentration of volatile fatty acids does is not always associated to their production and absorption rates, especially when the pH of the rumen differs.

1.2 Limitations, considerations and possible suggestions

The longer lactation period characterizing dairy goats than dairy ewes can be involved in the different response to the carbohydrates of the diets. Probably, in goats, the positive effect of digestible fiber on milk persistency can occurs in late lactation, when the requirements of the animals are lower. Thus, in this species it would be useful to test the effect of dietary carbohydrates also in advanced lactation.

The lack of effect of dietary carbohydrates on the hormonal status can be linked to the length of the observation period, probably not sufficient to have an evident tissue responsiveness. The length of the observation period was decided considering that heat stress would have been likely in late lactation, considering the seasonality of the

production of the ewes and of the goats in the Mediterranean environment. Heat stress notoriously affects the nutritional and hormonal response of the animals.

1.3 Conclusions

In conclusion, the different response to the carbohydrates of the diet (starch vs. digestible fiber) observed in mid lactating ewes and goats is probably linked to their differences in hormonal status, and in particular to their GH to insulin ratio, which in turn is probably associated to the differences in production level and genetic potential for milk production between the two species. Thus, it appeared that the type of carbohydrates of the diet could not change the hormonal status of the animals but, instead, the hormonal status of the animal differently modulated the productive responses to the carbohydrates of the diet. However, specific studies are needed to better understand if the differences observed in the hormonal status also affected the responsiveness of the tissues to the hormones.

1.4 Practical implications

The results of this dissertation gave preliminary indications about the best dietary combination of non-fiber carbohydrates and neutral detergent fiber to favor milk persistency in lactating sheep and goats during mid-lactation.

In sheep dairy farms, the use of diets rich in highly digestible fiber during mid-lactation will permit to:

- produce more milk, contrasting excessive body fatness;
- reduce feeding costs and the competition with nutrients than can be used also by humans, such as starch (Dann et al., 2014), since most feeds rich in digestible fiber are by-products (e.g. soyhulls, beet pulps, citrus pulps, wheat brans);
- reduce possible risks of sub acidosis linked to excessive use of starchy diets, improving the welfare of the animals;
- reduce the environmental impact of sheep dairy farms, since if milk production persistency is improved the cost of maintenance is diluted by the higher milk

production, with reduction of the greenhouse gas emissions and nitrogen excretions per unit of milk produced.

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