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CORSO DI DOTTORATO IN SCIENZE AGRARIE

Curriculum Scienze e tecnologie zootecniche

Ciclo XXXV

Intake and production responses of pregnant and lactating Sarda ewes fed hays of different composition and quality

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“Intake and production responses of pregnant and lactating Sarda ewes fed hays of different composition and quality”– Tesi di dottorato in Scienze Agrarie – Curriculum “Scienze e Tecnologie Zootecniche” Ciclo “XXXV” – Università degli studi di Sassari

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Intake and production responses of pregnant and lactating Sarda ewes fed hays of different composition and quality

A Thesis
Presented to the Doctoral School, Department of Agricultural Sciences
University of Sassari, Italy
In Partial Fulfilment of the Requirements for the Degree of
Doctor of Philosophy

by
Matteo Sini
2023

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"Intake and production responses of pregnant and lactating Sarda ewes fed hays of different composition and quality"– Tesi di dottorato in Scienze Agrarie – Curriculum "Scienze e Tecnologie Zootecniche" Ciclo "XXXV" – Università degli studi di Sassari

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FUNDINGS

La presente tesi è stata prodotta durante la frequenza del corso di dottorato in Scienze Agrarie dell'Università degli Studi di Sassari, a.a. 2022/2023 – XXXV ciclo, con il supporto di una borsa di studio finanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2014-2020 Asse III - Istruzione e Formazione - Obiettivo Tematico 10 "Investire nell'istruzione, nella formazione e nella formazione professionale per le competenze e l'apprendimento permanente".

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ACKNOWLEDGEMENTS

I would like to thank my mentor Prof Antonello Cannas for giving me the opportunity to embark on this path of professional growth. I thank him for spurring and guiding me with this enlightening 4 years of research, by letting me gain confidence and carry on the research work with freedom and critical thinking.

I also thank him for giving me the opportunity to be able to compare myself with the greatest experts in nutrition and having me participate in numerous international conferences. I would also like to thank Prof Alberto Atzori for encouraging and spurring me throughout my journey by giving me the opportunity to grow professionally. Also, I would like to thank the technicians who participated and gave me useful advice for my research activity and in particular Roberto, Antonio Fenu, Antonio Mazza, Alessandra and Emiliano. Finally, I would like to thank all my colleagues who have accompanied me on this journey and especially my fellow nutritionists, Fabio, Roberta, Margherita, Dimitrios, David, Lydia and Dr Ledda with whom I have shared the joys and sorrows of this long and challenging journey.

Finally, a special thanks to my Family who taught me a sense of duty, work and sacrifice, respect for others and the people around me, values that have given me the way to get here making me the person I am.

DEDICATION

This thesis is dedicated to my family

DECLARATION

I declare that this thesis and the results contained therein are my original thoughts and have never been submitted to another institution for consideration for a PhD or a degree of a similar nature.

Matteo Sini

GENERAL ABSTRACT

The quality of forage in modern dairy ewe farms has been showed to be a crucial and at the same time critical point for the nutritional management of the productive animals. Harvesting forage of high nutritional quality, low NDF and high CP and digestibility, may help producers in both covering the energy and protein requirements of the animals and in reducing production costs, buying less feedstuff from the market. This is particularly true for modern dairy sheep, which have high production levels but also high requirements and limited fermentative capacity.

Thus, this thesis, made of 5 chapters, focused on the importance of forage quality on intake and productive performances of ewes during late pregnancy and lactation.

Chapter 1 reviewed the literature, focusing on the role of the forage quality, and especially on fiber, in small ruminant nutrition and production. It has now been well established, especially in dairy cattle, that fiber plays a fundamental role on animal health, feeding behavior, voluntary intake, and productive performances of animals during all different physiological stages, and particularly during late pregnancy and lactation. Unfortunately, at present, there is far less information regarding the effects of fiber quality in small ruminants, and especially dairy sheep, compared to cattle, even though dairy sheep farms use proportionally larger quantities of harvested and fresh forages compared to cattle, and it is well known that forage quality is affected by many factors. Indeed, forage quality and digestibility depends on several specific plant characteristics and species, phenological cycles and maturation processes, technological harvesting aspects, such as haymaking, and the interaction of fiber with the whole farm system.

Chapter 2, aimed to clarify the role that forage quality plays in late pregnancy and early lactation of Sarda dairy ewes. An experiment was designed to understand to which extent forage quality affected voluntary intake, BW, BCS, and the energy balance of ewes during pregnancy and the subsequent early lactation. For that reason, 28 pregnant Sarda dairy ewes were divided into two groups, balanced for body weight, days in pregnancy, and expected litter number (mean±SD; BW, 54±8.7 kg; DIP 115±2; 1.24 litter number). The experiment started 30 days before the expected pregnancy and ended at 30 days of lactation. Two experimental diets were used during the pregnancy: **1**. Dehydrated chopped oat hay (OH) (9.6% CP, 65.6% NDF, DM basis) + 350 g/d of whole corn grains and 140 g/d of soybean meal; and **2**. Dehydrated chopped alfalfa hay of medium quality (AAM; 14.8% CP, 53.3% NDF, DM basis) + 440 g/d of whole corn grains. After lambing and during the first 28 days of lactation, all the ewes were fed the same diet, made by the pregnancy alfalfa dehydrated hay fed ad libitum, plus (440 g/d of whole corn grain + 135 g/d of soybean meal, DM basis). The diet fed during pregnancy affected the voluntary DMI (OH=1.161 vs. AAM=1.431 kg/d), the voluntary intake of hay (OH=0.702 vs. AAM=1.023 kg/d) and the NDF intake (OH=0.422 vs. AAM=0.588 kg/d) during this stage, while no effect has been observed on the same parameters in early lactation. Milk yield was numerically higher in AAMp group (OHp=2.345 vs. AAMp =2.477 kg/d) compared to OHp group. For the energy balance, the diet had an effect during pregnancy (OH= -0.520 vs. AAM= -0.120 Mcal/d) but not during early lactation. Combined, the data demonstrated that feeding alfalfa hay during late gestation increases the VDMI, the voluntary intake of hay and reduced the risks of ketosis leading to less negative energy balance. While the lack of differences in milk production particularly in the AAM group needs to be further investigated.

Chapter 3, had the objective to evaluate the effects of three different forages with different quality and composition on the productive performances of 21 Sarda dairy ewes in their mid-lactation (150 days in milk). Three groups balanced for DMI, milk yield (MY), body weight

(BW) and BCS, mean±SD; DMI 2.22±0.05 kg/d MY 2.00±0.03 kg/d; BW 53.9±2.04 kg; BCS 2.98±0.08, were created. One of the three diets were randomly assigned to each group: **1**. dehydrated chopped OH hay (OH; 7.3% CP, 63.9% NDF, DM basis) ad libitum + 176 g/d of whole corn grains and 360 g/d of soybean meal, DM basis. **2**. dehydrated chopped alfalfa hay of medium quality (AAM); 19.7% CP, 43.5% NDF, DM basis) + 528 g of DM/d of whole corn grains. **3**. dehydrated chopped alfalfa hay of high quality (AAH) (23.5% CP, 39.0% NDF, 43.3% ADF, 5.8% ADL, DM basis) ad libitum + 528 g of DM/d of whole corn grains. The diets affected the voluntary intake of hay, voluntary DMI (OH=1.289 vs AAM=2.085 vs AAH=2.733 kg/d, p<0.001), and NDF intake per day (OH=0.587 vs AAM=0.738 vs AAH=0.903 kg/d, p<0.001) and as % of BW. All were markedly reduced as forage quality decreased. Milk yield was also affected by the diets (OH=1.491 vs AAM=1.755 vs AAH=2.028 kg/d, p<0.001), being markedly reduced as forage quality decreased. The quality of the diet significantly affected the chemical composition of milk. This study highlighted the importance of feeding high quality forage during the mid-lactation phase to maintain the persistency of lactation and improve milk quality parameters.

Chapter 4, aimed to evaluate existing DMI prediction models by using the DMI data collected in the Chapter 2. Three DMI models (Pulina et al., 1998, Model 1; Gallo and Tedeschi, 2021, Model 2; and Serra, (1998), Model 3) for gestating and lactating ewes were compared to observed DMI, and evaluated using the software Model Evaluation System version 2.3.4. In addition, we integrated the model of Serra, (1998) with the continuous adjusted factor (fDMI) proposed by Gallo and Tedeschi (2021), developing Model 4. The results showed that Model 4 (Serra, 1998, adjusted) was the most precise and accurate in fitting the observed DMI in early lactation in both the AAM group (r^2=0.82, CCC=79, Cb=0.86 and RMSEP=0.20, P-value<0.001), and the OH group (r^2=0.91, CCC=79, Cb=0.83 and RMSEP=0.20, P-value<0.001). These findings suggested that more observed data are necessary to improve these models, including in them predictors associated to diet quality.

Chapter 5 reports the general conclusions and practical implications of this thesis.

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1 CHAPTER 1

The role of the fiber in the diet of small ruminants: a review

ABSTRACT

One of the fundamental aspects related to animal nutrition concerns fiber and its use in the diet of ruminants. Identified as one of the principal factors influencing animal response in both physiological and productive aspects, it has been a topic of study for thousands of researchers since the early 1800s. The reason why fiber assumes such a wide relevance in the ruminant nutrition research is due to the many factors that affect its quality and digestibility, such as several specific plant characteristics and species (for example those of grasses and legumes), phenological cycles and maturation processes, technological harvesting aspects such as haymaking, and the interaction of fiber with the whole system: feeds, technology, and animals. It has now been well established, especially in dairy cattle, how fiber plays a fundamental role on animal health, feeding behavior, voluntary intake, and productive performances of animals during all different physiological stages, and particularly during late pregnancy and lactation. However, at present, there is far less information regarding the effects of fiber quality in small ruminants. This review aimed to highlight all aspects of fiber utilization in small ruminants, with specific focus on sheep.

1.1 INTRODUCTION

Sheep and goats are large users of plant fiber (Van Soest, 1994; Mertens, 2002). Even though their similarity, small ruminants differ from large ruminants not only for their body size and weight, but also for the efficiency of utilization of fiber. Large ruminants can retain for a longer time than small ruminants the fiber in the rumen, due to their more advantageous fermentative capacity (i..e. rumen volume/maintenance requirements). In addition, they have an higher rumination efficiency, due to their strong mouth movements and also because they process the fiber more coarsely than small ruminants. Small ruminants compensate for these limitations with higher feed selectivity and higher concentrate digestibility, due to the fact they ruminate all particles, including concentrates, more finely than large ruminants (Poppi et al., 1981b,c; Van Soest 1994; Cannas and Van Soest, 2000). The ability of ruminants to ingest and digest forages is largely affected by their content of neutral detergent fiber (NDF) (Van Soest, 1994, Dado and Allen, 1995; Mertens, 2002a). Since the predominant part of ruminant diets is made by forages, there has been vast research worldwide to study fiber chemical and physical measurements methods, and fiber utilization by ruminants. Since in large ruminants the application and the knowledge around fiber is well established, the aim of this chapter is to review existing literature on fiber utilization in the feeding and nutrition of dairy sheep.

1.1.1 NDF and the quality of fiber

By a nutritional point of view, plant cell consists of a complex structure, made by cytoplasm and cell walls. Its most degradable and digestible components, such as sugars, starch, fat and proteins are found in the cytoplasm, or, in the case of pectin and other soluble fibers, mostly in the medium lamella. The insoluble and less degradable or undegradable fractions, such as hemicellulose, cellulose, lignin, and certain proteins, are instead part of the cell wall (Moore and Hatfield, 1994) and make what is often called "fiber", which indeed should also include pectin and other soluble fiber types. Fiber is currently measured with the Van Soest (1994) method, which includes the NDF, ADF and ADL fractions and substituted the much ess accurate crude fiber assessed with the Weende method. The NDF fiber represent the combination of hemicellulose, cellulose, and lignin, which are located in the cell wall of the plant and that have a structural function. The NDF fiber does not include pectin or other soluble fiber compounds, intentionally left out by the NDF, even though part of the fiber, because of their high degradability. NDF is obtained after a treatment with a combination of neutral detergent solution and α-amylase (aNDF). The latter is used to solubilize the refractory starch of the feeds. Because aNDF is often overestimated due to the presence of soil contamination, which can vary up to 5-10% on a DM basis. To avoid this contamination, subtracting the ash from the initial value of aNDF is possible to quantify NDF organic matter (aNDFom) (Mertens, 2002a, 2018). The ADF fiber, on the other hand, represents the part of cellulose, lignin and silicates after a treatment with acid detergent solution. ADL represents the portion of cell wall undegraded after ADF is treated with sulfuric acid. ADL, which is made mostly by lignin and cutin, is the complete indigestible portion of the plant (Van Soest, 1994). Hemicelleluose and cellulose can be estimated by difference as follows:

$$\text{Hemicellulose} = \text{NDF}-\text{ADF}$$

$$\text{Cellulose} = \text{ADF}-\text{Lignin}$$

The NFC, on the other hand, are the non-fibrous carbohydrates (Van Soest, 1994). They are made by sugars, starch and pectins, if present, are highly digestible and can be calculated as:

$$\text{NFC} = 100-(\text{CP}-\text{NDF}-\text{Ash}-\text{Fat})$$

This calculation is more accurate if from NDF is subtracted the CP fraction strongly associated to it, obtaining the NDFCPfree.

The NDF is made by at least two pools, which have a different degradation rate when fermented in the rumen. In fact, Huhtanen et al. (2006b) proposed that the NDF consist in two fractions, one slow and one fast, that are degraded by microbial enzymes at different rates. These fraction are nowadays the bases of the dynamic theory of 2 pools fiber degradation in the rumen (Mertens, 1973,1977; Van Soest, 2005; Raffrenato et al., 2018, 2019).

Factors affecting forage quality: plant and animal factors

Forage quality can be defined on the basis of ability of ruminants to eat, ferment and transform it into milk or meat (Ball et al., 2001; Newman et al., 2009). Forage quality can be expressed as the set of several variables, which depends on plant and animal factors, that collectively define its quality. The most important variables referred to the plant are stage of maturity, anti-nutritional factors, species difference, plant anatomy, cultivar, cellular composition and metabolism. The variables referred to the animal perspective are palatability, intake and digestibility (Conrad, 1966; Baumont et al., 2000; Katoch, 2022).

One of the most important plant factors affecting forage quality is the stage of maturity, that is influenced by the phenological phases. Ball et al. (2001) have shown that cool season grasses often have dry matter digestibility (DMd) above 80% during the first two to three weeks after growth begins in spring. Thereafter, digestibility decreases by 75% to 50% if we considered different parts of the plants (i.e. leaf vs steams). Crop maturity also affects feed consumption by the animals because as the plant mature and become more fibrous and lignified, it also reduces its CP content, and thus forage intake and digestibility decreases (Figure 1) dramatically (Kilcher and Troelsen 1973; Jung and Allen 1995; Khorasani et al., 1997).

Maturity stage has also shown to be a major factor influencing the nutritional quality of forages. Katoch et al. (2012) observed that in early growth stages, tall fescue grass had high CP and in vitro dry matter digestibility (IVDMD) and low NDF, ADF and hemicellulose content, while advancing in maturity decreased its CP and IVDMD. Comparative results have agreed with previous results when investigating alfalfa and timothy grass at late bud and pre-bloom stages (Yu et al., 2003).

Differences between species (grasses vs. legumes) also affects the forage quality. Legumes generally produce higher quality forages than grasses because they are generally richer in fiber of higher digestibility especially on the leaves, that are easily attacked by cellulolytic bacteria and digested at ruminal level (Wilson 1990,1993; Van Soest 1994; Ball et al. 2001). Moreover, it has been shown that alfalfa leaves in all stages of maturity tend to have a constant concentration of NDF and ADF contents (Katoch et al., Unpublished data from Katoch, 2020). This suggests that approaching the maturity stage, differences in NDF contents in legumes is more pronounced on the stem than on the leaves.

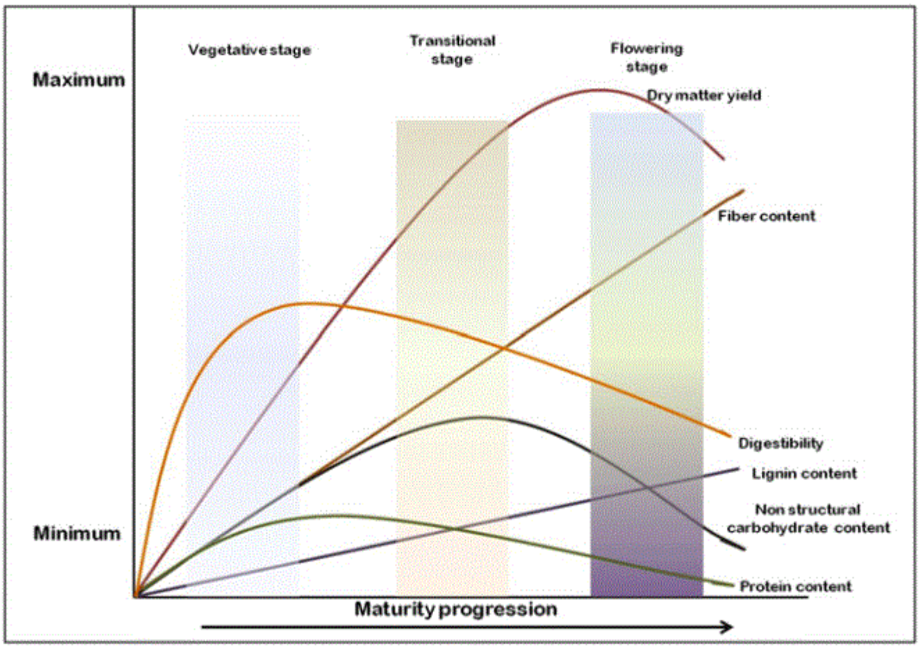

Figure 1. Effect of maturity on quality of forage grasses and legumes (Katoch, 2022).

Forage quality can also be evaluated considering animal factors. They include palatability, defined as “the sum of all physical and chemical characteristics of a feed that evoke appetite, such as olfactory, gustatory and tactile stimuli during foraging and chewing” (Baumont, 1996) is influenced by texture, leafiness, fertilization, and moisture content (Ball et al., 2001, Katoch, 2020). Generally, high quality forages tend to have high palatability; however, there could be differences among animal species. For example, it has been well established that sheep prefers more legumes than grasses, while goats prefer more grasses and bushes than legumes (Van Soest, 1994). Typically, the higher the palatability and forage quality, the higher is the intake. Another animal factor affecting forage quality is its digestibility, which indeed is a combination of plant and animal factors and varies widely. For instance, immature plant tissues can be digested up to 80-90%, while less than 50% of mature stem material is digested (Ball et al., 2001, Katoch, 2020). Whole tract forage digestibility depends mostly on its rumen degradability, which is the result of the "competition" between their degradation rate and their passage rate through the animal's digestive system.

1.1.2 NDF digestibility

Over the last five decades, there has been a vast research on the factors affecting NDF digestibility and its interaction with time of fermentation (Mertens, 1973, 1977, 1993; Mertens and Ely, 1979; Van Amburgh et al., 2003; Ellis et al., 2005; Van Soest et al., 2005; Raffrenato and Van Amburgh, 2010; Raffrenato et al., 2009, 2018, 2019). These authors demonstrated that the laboratory method to describe NDF was not a good predictor of fiber digestibility. Indeed, different plants can possess similar NDF concentration but can have largely different fiber digestibility and can induce very different DMI and milk production, where the highest animal responses were achieved with highest levels of NDF digestibility (Grant et al., 1995; Dado and Allen, 1996; Oba and Allen, 2000).

For this reason, an approach based on the partitioning in NDF subfractions, to be measured with in vivo or in vitro methods, has been devised (Allen and Mertens, 1988; Huhtanen et al., 2006a). These fractions are digestible NDF (dNDF), potentially digestible NDF (pdNDF) and indigestible NDF (iNDF). The dNDF represent the amount of NDF, fast and slow pool, that is digested by the animal. However, since the total retention time for each feed is finite, not all the NDF is digested because of the passage and retention time of the feed material through the rumen. Potential digestible NDF (pdNDF) describes the amount of NDF that could be potentially digested in a certain amount of time, generally after no more than 240h, while the iNDF is the fraction that is totally indigestible by the rumen microbes because of the attached lignin, even if the feed stays in the rumen for infinite time (Lucas, 1964; Van Soest, 1994; Mertens, 1993; Huthanen et al., 2006). The usual approach to calculate iNDF by the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992; Fox et al., 2004; Tylutki et al., 2008) is by multiplying the ADL content for a fixed factor (2.4), so that iNDF= ADLx2.4/NDF (Chandler et al., 1980; cited by Cotanch et al. 2014).

More recently the new term undigested NDF (uNDF) was introduced by Mertens (2013), to indicate the iNDF fraction estimate by long fermentation times and not calculated .

1.1.3 Regulation of forage voluntary intake in small ruminants

Regulation of voluntary dry matter intake (VDMI) in ruminants has been deeply reviewed on the last century. Forage VDMI has been studied for its important effect on driving the level and the efficiency of ruminant production (Waldo, 1986; Allison, 1985; Allen, 1996). However, since VDMI is influenced by several variables, it is still a subject of great interest in ruminant feeding and nutrition. In fact, VDMI is the animal's expression to a complex mechanism of neurological, chemical and physical stimuli involving the animal's response to the administered diet and environment. Mayer (1953) was one of the first to hypothesize the involvement of the blood and neurological control on VDMI, while Conrad et al. (1964) was one of the first to hypothesize VDMI is controlled by the interaction of physical and chemical factors.

More recently this theory was also supported by other researchers that agreed and gave more evidence about the factor affecting VDMI, including a deeper knowledge on neurological and metabolic interactions (Forbes, 1980; Baile and McLaughlin, 1987; Fisher et al., 1987; Ingvartsen and Anderson, 2000, Allen and Bradford, 2009). As in other ruminants, intake in small ruminants is under the regulation of 1) metabolic control and 2) physical control. When the animal eats forages and other feeds of high quality and digestibility and satisfy its energy demand, usually limits its intake due to the metabolic control of ingestion. However, when the animal eat forages or other feeds of low quality and digestibility, it tries to consume more forage to reach a certain amount of energy and satisfies its energy requirements, however it is limited by the fill effect of the forage (Blaxter et al., 1961). This theory was confirmed by a study in sheep which were fed with two different cuts of grass hay (early vs late cut) and a clover hay. DMI and digestibility were higher for the hays with lower NDF, i.e. early cut grass and the clover, compared to that with high NDF., the late cut grass hay, confirming that lower NDF content allowed the animals to eat higher amount of forages (Aitchison, 1986). Forages of low quality have high NDF concentrations, which make them more bulky at rumen level, thus limiting feed intake due to the fill factor (Van Soest, 1965; Mertens, 1987, 1994a; Allen, 1995). It has also been shown that large ruminant have a better ability to adapt to bulky diets compared to small ruminants (Van Soest, 1994). Large ruminants reach satiety at higher levels of NDF content than those achieved by small ruminants, and this might be because of the higher rumen and gastrointestinal tract (GIT) volume to requirements ratio typical of large ruminants (Van Soest, 1994). When rumen fill is reached, the ruminants stop to eat not because they covered their energy requirements but because they cannot contain more fiber in the rumen (Lehman, 1941; Waldo, 1986; Forbes, 1995). Finally, Forbes (1986), in concordance with Blaxter et al. (1961), Blaxter and Wilson (1962), Van Soest (1965) noticed that as the digestibility increases, the fill effect control tends to decrease, probably because the high supply of energy from highly digestible forages determines a prevalence of the metabolic intake control mechanism over the fill control. Furthermore, a more digestible forage might pass the reticulorumen faster than a less digestible forage, by lowering the mean retention time (MRT) in the rumen (Campling et al., 1961; Freer and Campling, 1965; Thornton and Minson, 1972, 1973; Laredo and Minson, 1975).

Several other studies have shown that the rate of passage is inversely correlated to digestibility and that forage intake is controlled from rate of passage at certain levels (above 66%) of digestibility (Conrad, 1966; Blaxter and Wilson 1962). Montgomery and Baumgardt (1965b) further reported that the particle size and the leaves/stem ratio controls GIT fill. However, MRT in the rumen can vary widely depending on the intake level, forage sources (grasses vs. legumes), content of NDF, feed density and the particle size of the forage (Mertens, 1980). In few studies conducted in sheep fed hay ad libitum, it has been noticed that the mean retention time in the reticulo-rumen (MRTRR) of 1 mm long particles with a density of approximately 1.0 g/ml was about 67 h, that is eight times longer than the MRTRR of fluid; the heavier particles were retained only three times longer than fluid. Thus, MRT is influenced more by the density and degree of hydration of the particles than to the particle size per se (Kaske and Engelhardt, 1990) However, considering the digesta passing through the reticulorumen orifice, long particles (10mm) must be reduced to at least 4 mm by rumination (Kaske and Engelhardt, 1990). In another sheep study, MRT decreased during pregnancy by 14-32% and increased up to 15% in early lactation (Kaske and Growth, 1997). These authors contend that MRT decrease during pregnancy and increase during lactation is a consequence of an increase in rumen volume and DMI. Other studies underlined that shorter MRT were associated with higher intake of leaves vs. steam in legumes and grasses, respectively (Hendricksen et al., 1981; Poppi et al., 1981b). Poppi et al. (1991c) observed that as legume intake increased, there was a decrease the MRT, while the rate of digestion increased. In the same study, higher rate of passage was due to a higher digestibility of leaves compared to steams (Poppi et al., 1991c). In addition, Poppi et al. (1981b,c) found that sheep were found to retain NDF in the rumen for a shorter time than cattle, and this difference also was associated with a higher rate of digestion of the NDF and more rapid rate of passage of the NDF. Tedeschi and Fox (2020) summarized the factors affecting VDMI as the fill and metabolic effect. Adapting the model of Mertens (1987) for dairy cows and that of Cannas et al. (2016a) for lactating sheep, they showed (Figure 2A and 2B, respectively) that the relationship between DMI expressed as percent of BW and the dietary NDF expressed as percent of DM regulated by fill intake and metabolism in dairy cows (Figure 2A) and dairy ewes (Figure 2B) for different production levels. The figure identifies the possible DMI when intake is regulated by the energy demand (blue area) or when intake is restricted by fill effect (green area), while the DMI above the energy demand but not restricted by fill effect is represented by the brown area. Optimum levels of dietary NDF are represented by the intersection of the green and blue line of a certain production level (Tedeschi and Fox, 2020). As an example, for sheep that produce 2 kg/d of milk the optimal level of DMI (as % BW) and dietary NDF concentration (DM basis) are 4.61% and 45.6%, respectively (Figure 2B; Tedeschi and Fox, 2020).

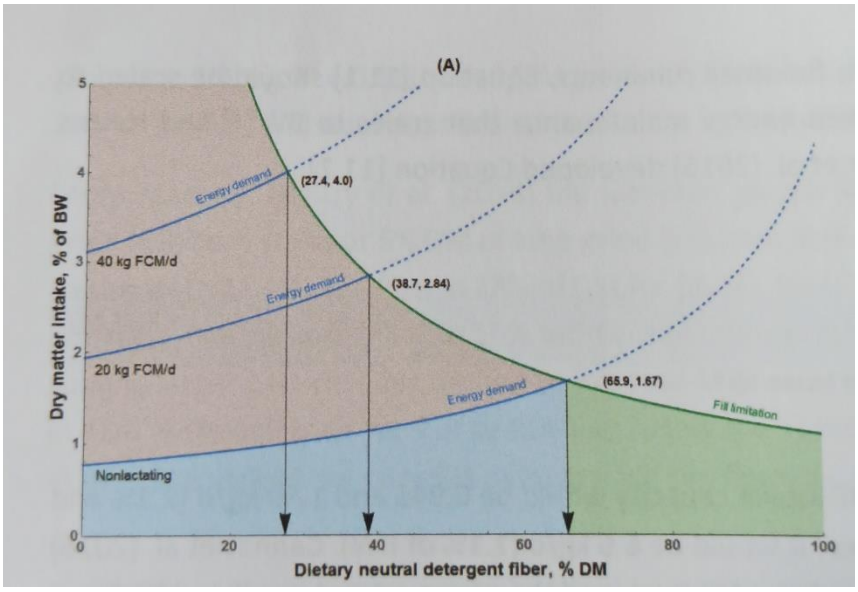

Figure 2(A). Relationship between dry matter intake (DMI, %BW) and dietary NDF (NDF, %DM) regulated by fill or metabolism effect in dairy cows. Tedeschi and Fox (2020) adapted from Mertens (1987).

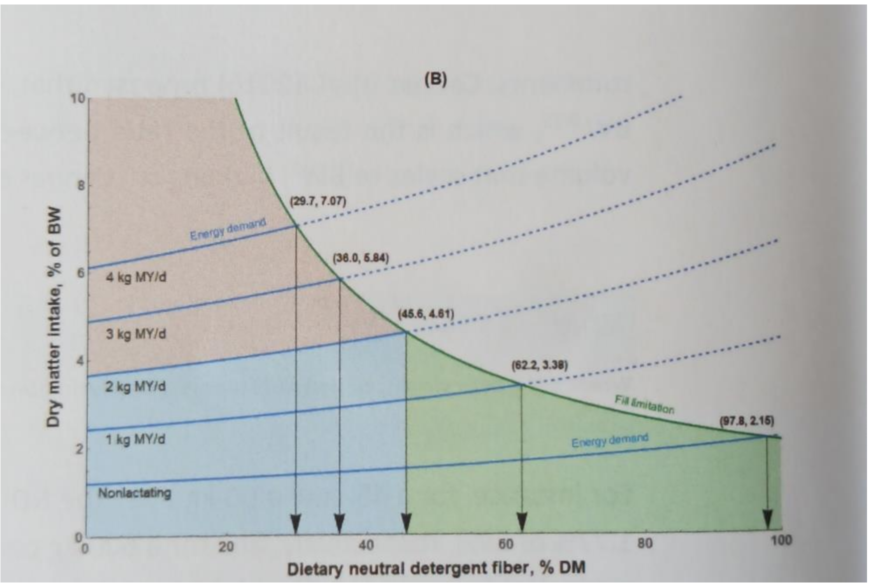

Figure 2(B). Relationship between dry matter intake (DMI, %BW) and dietary NDF (NDF, %DM) regulated by fill or metabolism effect in dairy ewes. Tedeschi and Fox (2020) adapted from Cannas et al. (2016).

1.1.3.1 Effects of forage particle size and physically effective fiber (peNDF)

One of the variables that affect VDMI is particle size. Forage particle size is able to influence chewing activity (eating and rumination), ruminal pH and milk fat percentage in dairy cattle (Balch, 1971; Donefer, 1973; Van Soest, 1994; Allen, 1997; Grant, 1997; Varga et al., 1998) and in dairy sheep (Jalali et al., 2012; Helander, 2014).

It has been well established that to leave rumen the feed particles must be reduced to a size, i.e. they should be able to pass through a sieve of 1.18-mm, which is considered to be the critical threshold size to pass the reticulo-rumen orifice in sheep and goat (Reid et al., 1979; Poppi et al., 1985; Domingue et al., 1991). This theory was confirmed in a study conducted on sheep: when chopped silage was offered instead of long, sheep increased the VDMI as a consequence of a decrease in MRT in rumen (Welch, 1967; Dulphy and Demarquilly, 1973; Dulphy et al., 1975; Laredo and Minson, 1975; Deswysen et al., 1978).

Information about particle size is important, as it affects the rumination pattern in ruminants. Domingue et al. (1991) observed that when sheep and goats were fed the same alfalfa hay, goats spent more time eating (+3 h) and less time ruminating (-2h) than sheep. This aspect reflected the inefficiency on chewing activity of sheep that need more time to ruminate and reduce particle to size smaller than <1.8-mm.

It has been demonstrated that when sheep and cows are fed the same diet made of: 1) high quality dehydrated ryegrass 2) medium-quality dehydrated ryegrass and 3) mix of medium-quality ryegrass and 40% of barley grains, in three different physical forms: long particles (size >50 mm), grounded or pelleted (higher DMI was observed in sheep compared to cows when the pellet form was supplied, increasing the DMI of 45% in sheep and only 11% in cows within the diet made of medium-quality dehydrated ryegrass (Greenhalgh and Reid, 1973). In addition, intake expressed as g/kg of metabolic weight as increased more in in younger than adult animals. The reason for this is that the decrease in particle size helped young animals to eat proportionally higher amount of feed, because they were more limited by larger particles (lower rumination efficiency) and have to eat more, per kg of BW, to satisfy their metabolic requirements compared to bigger animals.

Cannas (1995) studied the effects of particle sizes on the feed intake, milk production and quality and ruminal pH in Dorset, Finnish and Dorset x Finnish sheep breed. In the trial, ewes were fed three diets with different particle sizes (grinding with a 1 mm, 2.4 mm, and 12 mm sieve), while the ration consisted of 55% of grass forage and concentrates. The results showed that the diets containing the smallest particle sizes (1 and 2.4 mm) had a tendency to increase DMI and milk production, without changing the milk composition and altering the digestibility of the DM. Furthermore, while the time spent eating was higher in diets containing 1 mm particle sizes compared to their counterparts, the rumination time was higher for the diets containing the largest particles (12 mm).

Recent studies conducted on fattening sheep and goats have attempted to improve the knowledge on particle size in small ruminant diets. In an experiment, sheep, goats and llamas were fed chopped (6 mm) grass hay (GH; 58% NDF) and chopped (6 mm) grass seed straw (GSS; 81% NDF). The results showed that DMI was higher for sheep and llamas fed the GH diet compared to GSS diet. In addition, the mean time spent eating, chewing and ruminating, expressed in kg/DM and kg/NDF was higher when GSS diet was fed (Jalali et al., 2012). When Iranian male sheep were fed two diets differing in lucerne particle size (2.38 mm and 0.94 mm), respectively, an higher rate of degradation of DM and NDF was observed for the 0.94 mm particle size diet. In contrast, mean ruminal pH was higher in the 2.38 mm diet than in the 0.94 mm diet (5.86 vs. 5.76) (Alamouti et al., 2009).

Similar results were reported in a study conducted on fattening male goats, in which a complete pelleted diet made of straw differing in particle size (4 mm vs. 8 mm) was supplied (Khurshid et al., 2023). Also, fattening lambs of 37 kf of BW fed with Tifton hay plus a commercial pellet with four different particle size ((2mm, 5 mm, 10 mm, and 25 mm), showed lower rumination and chewing activities when fed 2 mm particle size rations (Gomes et al., 2012).

Physically effective fiber (peNDF) has been defined as the physical properties of a feed that stimulate total chewing activity and create the biphasic stratification of ruminal digesta (Mertens, 1997).

The importance of physical properties of a feed is so important that different methods to asses it were developed, from the one of Balch (1971) to that of roughage value index (RVI) of Sudweeks et al. (1979, 1981), to those of Norgaard (1986) and the theory of roughage value unit (RVU) of Mertens (1986). Mertens (1997) for the first time combined the chemical and physical properties of fiber on a single measure. Mertens (1997, 2002b) put on the basis for the modern formulation of rations, in which an elaboration on the minimum fiber requirement of ruminants to maintain ruminal function, health, productivity and welfare was documented. One of the novelty aspect of studies from Mertens (1997, 2002b) was the distinct difference between the peNDF and effective fiber (eNDF). The peNDF referred to the physical property of a feed in stimulating chewing activity, while the eNDF can be defined as the ability of a feed to replace roughage to maintain a constant level of milk fat percentage (Clark and Armentano, 1993; Swain and Armentano, 1994). The peNDF is always less than the NDF, while eNDF can be smaller or bigger than the NDF concentration (Mertens, 2002b).

Mertens developed the methods to measure the physical effectiveness factor (pef: all particles retained in a 1.18 mm sieve) and the peNDF (peNDF = NDF% x pef), where pef is considered equal to 0 in feeds that do not stimulate chewing activity and assumes maximum value of 1 when the feeds promote the maximum chewing activity (Mertens, 2002b).

As aready said, the pef is also referred to "the fraction of particles that are retained on the >1.18-mm sieve after dry vertical sieving" (Grant, 2022). To support this theory, Mertens (1985a, 1986, 1997, 2002b) and Poppi et al. (1985) previously observed that particles >1.18-mm are resistant in passing the reticulo-rumen orifice and promote rumination. In contrast, particles <1.18-mm do not stimulate chewing activity and are not able to maintain adequate ruminal function. In a metanalysis conducted on 45 experiments, Mertens (1997) noticed that there was a positive relation (r2=0.71) between ruminal pH and peNDF, and that to maintain constant ruminal pH at 6.0, the dietary peNDF must be 22% of DM.

A similar pattern was observed between peNDF and milk fat concentration of dairy cattle. To maintain a 3.4 milk fat percentage it is necessary to have at least of 20% peNDF, DM basis (Mertens, 1997, 2002b). In agreement, Fox et al. (2004) reported that 24.5% of peNDF on a DM basis was the optimum to maintain stable ruminal pH and milk fat percentage, while Zebeli et al. (2008) found an optimal value of peNDF of 30-33% (DM basis).

The laboratory methodology of Mertens to sieve diets has been applied in the field by using the Penn State Particle Separator (PSPS) (Lammers et al., 1996) and the ZBox (Cotanch et al., 2006). The PSPS is widely used at farm level and can be considered as a field measurement to estimate the peNDF of wet total mixed rations (TMR). The PSPS estimates not only the peNDF but also other particle sizes and fractions, by using three different sieves (19 mm; 8 mm; 4 mm) and a bottom contained that collects particles < 4 mm.

1.1.4 Animal factors affecting forage voluntary intake

Besides the physical and energetic factors that are most associated with the feed and the neurological and metabolic systems, there are other factors that can control voluntary intake. Body weight of the animal, species, physiological status (pregnancy and early lactation) and body condition are among the most important animal dependent factors.

1.1.5 Body weight and body size

Earlier studies on 18 species of ruminants showed that BW was highly and positively related (r2=0.98) with the DM content of the rumen (Illius and Gordon, 1991), and that the GI tract volume was also associated BW (Illius and Allen, 1994; Demment and Van Soest, 1983). This is because as body mass increases, both rumen volume and requirements increase. However, NDFI is not always associated to BW, because of the bulky effect of the fiber. Therefore, when forage is fed, there is a linear relationship between BW and NDF intake when intake is not limited by the energy concentration of the diet, while there is a NDF intake decrease when the potential intake is limited by its energy concentration (Van Soest, 1994).

Since the growth of rumen volume in ruminants is directly proportional to their BW (Demment and Van Soest, 1983) but the maintenance requirements are proportional to BW0.75 , the so called metabolic weight (Waldo, 1969; Klieber, 1961), small ruminants have a smaller fermentative capacity and have to eat more (as percentage of BW) than cows to compensate for this, because small ruminants adopt the strategy of eating more (by speeding up rumen passage rate), as proportion of BW, degrading only the dietary fractions with highest degradability (Cannas, 2000). In fact, high-producing sheep generally have higher levels of intake (4.0-6.0% of BW) than cows (Avondo and Bordonaro, 2002; Cannas, 2004). The same pattern is reflected when the level of intake of NDF (NDFI, % of BW) is considered. Indeed, Mertens (1987) reported for lactating dairy cows an optimal level of intake of NDF around 1.2% of BW, while Cannas et al. (2016b) reported a range between 1.7-2.1 % of BW in lactating sheep. Similar values were observed by Molle et al. (2014,2016) and Olsen (2016). This explains largely why sheep have less time to retain forages in the rumen (Cannas and Van Soest, 2000).

Consequently, when fed the same diet, small ruminants have a lower digestibility of fiber than cattle. To compensate for their lower fermentative capacity, sheep also adopt a highly selective feeding behavior, selecting the most digestible plant parts. This is allowed by their narrow muzzle and by the motility of their lips. In goats, this selective behavior is even more clearly distinct than in sheep, when they are managed in the same environment (Leclerc, 1985; Van Soest, 1994; Cannas, 2004).

Voluntary DMI also depends on animal age. Young animals, including lambs (5 month of age) consumed more digestible and proteic diet, with less fiber content compared to mature sheep due to their but also because their higher energy requirements per kg of BW (Arnold, 1981).

1.1.5.1 Physiological status: late pregnancy and early lactation

Physiological status is a fundamental driver of VDMI during the last month of pregnancy and in early lactation. Various factors influence VDMI during these stages (Cannas et al., 2002, 2016).

Late pregnancy

During late pregnancy VDMI decreases due to abdominal cavity space occupied the growing conceptus (Hutton, 1963; Arnold, 1970b; Allison et al., 1981, Allison, 1985).

Cannas et al. (2016b) studied the differences between small and large ruminants, explaining that sheep and goat often sustain a bigger effort in terms of energy requirements during pregnancy compared to cows. This because sheep compared to cows are more prolific and have a shorter pregnancy. Thus, the daily growth rate of the fetus (expressed per kg of BW of the mother) in the last month of pregnancy is 4 times higher in sheep carrying twins than in cows with singles, and 6 times higher when carrying triplets. This combined with a high space occupied by the uterus in sheep with twins or triplets. Thus, diets made mostly by forages can be very challenging for sheep

A study conducted in 2 consecutive years by Orr and Treacher (1984) reported that when pregnant sheep were fed ryegrass hay ad libitum (10-15cm length) and five levels of concentrate (0; 150; 450; 750 and 1050 g/d), forage intake decreased with higher levels of concentrate and decreased more in sheep carrying triplets than those carrying twins. This perfectly explains that as the levels of concentrate increase, sheep covered more of their pregnancy energy requirements and did not need much forage to satisfy their requirements. On the contrary, as reported by the same authors, in year 2, when sheep were fed solely hay, they increased their intake and sheep carrying twins increase hay intake more than those carrying triplets. This also explain that when sheep are fed only with forage source diet, they tended to increase forage intake to satisfy their pregnancy energy requirements, to the point the reached the maximum rumen fill; this also might explain why sheep carrying twins had an higher intake than those with triplets, more limited by the space occupied by the uterus..

Macedo Junior et al. (2012) observed, in pregnant sheep fed restricted and unrestricted diets in terms of energy and protein requirements, that ewes with two fetuses had higher DMI during pregnancy than the single lamb ewes, considering the restricted diet, probably due to the increase in energy requirements caused by the two fetuses. On the contrary, the NDFI (% of BW) was higher both in restricted and unrestricted ewes with single fetus compared to twins, while within the ewes carrying twins, the restricted group had higher NDFI (% of BW) than the unrestricted one. These results are in concordance with the fact that: a) during pregnancy the fetus compresses the rumen and limits the potential intake of the sheep, and b) in that case of restricted diets, the feed regime influences the sheep with twins, forcing them to increase as much as possible the intake of NDF, to achieve the highest level of energy intake from the diet. As reported in this review, Mertens (1987) identified an optimal level of NDFI intake of 1.1-1.2% of BW. The results of Macedo Junior et al. (2012) showed values like those of Mertens (1987) for pregnant ewes carrying twins, which ranged from 1.01% to 1.66% of NDFI. Similar results have been documented by Helander et al. (2014), who reported for late gestating ewes values of NDFI of 1.17-1.24% of BW, when fed un-chopped silage, chopped silage and concentrate supplied administrated separately, and a mix of chopped silage mixed to concentrates. Similar values of NDFI as % of BW have been also documented by Olsen et al. (2016).

All the data reported above were obtained in meat sheep, most of them with high body weight. Instead, little data are available for the late pregnancy of dairy ewes of small size, as the Sarda breed.

Early Lactation

It has been documented that body reserves can affect VDMI and performances during pregnancy and early lactation (Forbes, 1969). Body reserves can be measured by the body condition score (BCS). Sheep with too high levels of body reserves during pregnancy may have a reduction in VDMI, and subsequent immunodepression, increase in ketone bodies in the blood with risks of ketosis and low milk production during lactation (Lacetera et al., 2001, 2002; Norgaard et al., 2008; Schlumbohm and Harmeyer, 2008; Karagiannis et al., 2014). In fact, when sheep are too fat, leptin, an anorexic hormone synthetized by the fat tissue, reduces the appetite (Norgaard et al., 2008). Considering this, when a sheep is obese at lambing, there is a double problem; a) just after lambing the animal needs time to restore his appetite, and b) if the leptin acts on the appetite, the animal reduces its intake, thus ketosis may appear (Norgaard et al., 2008; Cannas et al., 2016b). Similarly, sheep with BCS <1.75 at lambing produced a 30% less milk in the first 9 weeks of lactation compared to ewes with higher BCS (Atti et al., 1995), possibly because thin sheep did not have enough body fat to mobilize as energy source to sustain milk production.

Given that from1 month after lambing sheep restores their potential intake (NRC, 2007), it is crucial to properly plan the nutritional management of pregnant ewes during late gestation to allow them to better perform in lactation. To do that, more information is needed on the optimal fiber intake in sheep, due to the lack of data on this issue. Cannas et al. (2002, 2004, 2016) were the first to propose recommendation on fiber requirements during the lactation of sheep, suggesting optimal dietary NDF concentrations and corresponding DM intake in ewes fed forages (grass and legumes) and concentrates at different production levels. The values reported were calculated assuming that the optimal NDF concentration is that that maximizes fiber and DMI, with optimal values that change depending on milk production and BW.

1.2 Conclusions and future perspective

To conclude, the importance of fiber in small ruminants' nutrition is paramount to ameliorate their production capacity and longevity. Fiber is an essential component of animal feeds, and this review highlighted some factors affecting voluntary fiber intake, voluntary dry matter intake and the effect of forage sources on their rumen mean retention time. Furthermore, this review highlighted the importance of particle size and peNDF, particularly for small ruminants. However, this review emphasized the fact that most of the studies and the theories, were conducted and developed based on large ruminants' data. There is a general lack of information about the utilization and effects of forage quality in the diets of small ruminant. This is certainly due for the importance that large ruminant has in the world social and economic development, being largely responsible of the demographical growth.

Most of the studies conducted in small ruminants are based on meat sheep and much less research focused on dairy ewes. However, worldwide there in an increasing interest in dairy milk ewes and goats and an increasing demand of small ruminants' products, i.e., milk, cheese and meat.

The implementation of the new theories regarding the chemical and physical characteristics of feeds, a special forage, in mechanistic models have enabled nutritionists to formulate rations more accurately than ion the past.

Certainly, more information is needed for the very highly critical stages, such as late pregnancy and lactation, so that optimal nutrient recommendations, especially for dietary NDF, could be proposed.

Thus, the PhD project described in this dissertation has focused his interest to the impact of forage quality on the nutrition and production of dairy sheep.

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2 CHAPTER 2

Effects of high and low quality forages during the transition phase in dairy Sarda ewes

ABSTRACT

Late pregnancy and early lactation are very critical stages for ewes, due to their high requirements and low voluntary dry matter intake, which might lead to metabolic disorders during pregnancy and subsequent low milk production. The utilization of high-quality forages might limit these risks. Herein, this study aimed to evaluate the critical variables on the effects of two forages of different quality, supplemented with fixed doses of concentrate, on voluntary dry matter intake, forage intake, NDF intake, body weight, body condition score, energy balance, BHB, milk production and milk quality, during late pregnancy and subsequent early lactation of Sarda ewes. Twenty-eight pregnant Sarda dairy ewes were divided into two groups, balanced for body weight, days in pregnancy, and expected litter number (mean±SD; BW, 54±8.7 kg; DIP 115±2; 1.24 litter number). The experiment started 30 days before the expected pregnancy and ended at 30 days of lactation. The two experimental diets used during the pregnancy phase were: 1. Dehydrated chopped oat hay (OH) (9.6% CP, 65.6% NDF, 4.9% ADL, DM basis) and a fixed supplementation of 350 g/d of whole corn grains and 140 g/d of soybean meal; and 2. Dehydrated chopped alfalfa hay of medium quality (AAM; 14.8% CP, 53.3% NDF, 8% ADL, DM basis) and a fixed supplementation of 440 g/d of whole corn grains. After lambing and during the first 28 days of lactation, all the ewes were fed the same diet, made by the pregnancy alfalfa dehydrated hay fed ad libitum, plus a fixed concentrate supplementation (440 g/d of whole corn grain + 135 g/d of soybean meal, DM basis). A factorial design with feeding treatment, time and their interactions as fixed factors and animals as random factor was applied. The diet fed during pregnancy affected the voluntary DMI (OH=1.161 vs. AAM=1.431 kg/d), the voluntary intake of hay (OH=0.702 vs. AAM=1.023 kg/d) and the NDF intake (OH=0.422 vs. AAM=0.588 kg/d) during this stage, while no effect has been observed on the same parameters in early lactation. Milk yield was numerically higher in AAMp group (OHp=2.345 vs. AAMp =2.477 kg/d) compared to OHp group. Milk fat content but not milk protein content, were also affected by the diets fed during pregnancy. Regarding the energy balance, the diet had an effect during pregnancy (OH= -0.520 vs. AAM= -0.120 Mcal/d) but not during early lactation. Combined, the data presented above demonstrated that feeding alfalfa hay during late gestation increases the VDMI, the voluntary intake of hay and reduced the risks of ketosis leading to less negative energy balance. However, this feeding regimen did not affect milk production during early lactation. This suggest that feeding low quality diet during late gestation does not clearly affect the performances and milk production in early lactation regardless of being exposed to high quality diet. Therefore, the lack of differences in milk production particularly in the AAM group needs to be further investigated.

Key words: **Late pregnancy, early lactation, forage, Voluntary DMI, NDF**

2.1 INTRODUCTION

The transition from pregnancy to lactation is one of the most critical physiological phases in animals' life. This is particularly true for sheep and goats. Even though small ruminants are often considered by field nutritionists specialized on cattle as cows 10 times smaller, and consequently with 10 times lower requirements, maintenance energy and protein requirements are not proportional to body weight but to metabolic body weight (BW^0.75). This means that compared to sheep and goats, cattle 10 times heavier have solely 6-7 time more maintenance requirements but a rumen volume, which grows proportionally to body mass, 10 times bigger (Van Soest, 1994). This suggest that cows have more rumen volume for unit of maintenance requirements and can thus eat, if needed, low quality, bulky forages to cover their requirements and are, therefore, less challenged than small ruminants by low quality diets. Furthermore, small ruminants have much shorter pregnancies and thus the foetus has to grow faster compared to that of cows, with very high expenditure of energy and protein in the last month of pregnancy. Considering that sheep and goats are much more prolific than cattle, the total litter weight as proportion of the mother's weight is usually much higher in small ruminants than in cattle (Cannas et al., 2016).

In the last 50-60 days of gestation, the growth rate of the foetus increases rapidly, and the uterus increase its volume, compressing the rumen space. Consequently, the animal decreases their voluntary intake as a result of the limited space available in the rumen to reach the optimal intake. Due to this physiological problem, it has been reported that sheep are able to increase the rate of passage of the digesta through the rumen during pregnancy, to increase the turnover of the nutrients and increase the intake of digested nutrients (Forbes, 1968; Kaske and Growth, 1997, Macedo Junior et al., 2010; Benevides et al, 2011).

Due to these limitations, a crucial aspect during the transition phase is to supply high quality forages, with high digestibility and low rumen fill, due to their high degradation rates of fiber and their low linin content. Recently, several experiments have reported that the use of high quality forages fed alone or mixed with concentrate well supported sheep during pregnancy and early lactation, increasing their feed intake, maintaining their BW and BCS and controlling their β-hydroxybutyrate (BHB), limiting thus the risk of ketosis (Nadeau and Arnesson, 2008; Bernes and Stengarde, 2011; Helander et al., 2014). High quality forages fed during late pregnancy might have positive repercussions during early lactation performances, since they favour and adequate development of the mammary gland (Norgaard et al., 2008).

The study reported above were conducted with Nordic ewes of very high BW and body mass (around 90 kg). However, no much information is available for the Sarda dairy ewes, which are half in body mass (around 45-50 kg of BW) compared to the Nordic breeds and thus, from the mechanism explained above, have less rumen volume per unit of maintenance requirements compared to large boy mass breeds. For his reason and with the aim of defining the optimal and maximum rumen NDF fill of Sarda ewes during the transition stage, we compared forages of different quality during the late pregnancy and early lactation of Sarda.

2.2 MATERIAL AND METHODS

Two experiments were carried out over three consecutive months, December 2021-February 2022, at “Stalla didattico-sperimentale Mauro Deidda” (40° 46’N, 8° 29’E, altitude 89m above sea level), University of Sassari, Department of Agricultural Science, Sassari, Italy. Experimental procedures used in the study were approved by the Research Animal Ethics Committee of the Universiy of Sassari.

2.2.1 Animals, feeding and experimental design

The experiment included 28 late gestating Sarda ewes, divided in two groups, which were balanced for BW, days in pregnancy (DIP) and expected litter number. Each group consisted of 14 ewes allocated to one of the two dietary treatments four weeks before parturition. After lambing and during the first 28 days of lactation, all the ewes were assigned to the same dietary treatment. The experiment started at 115 days (s.d. ±2) in pregnancy and it was concluded at 28 days after lambing. During the experiment, the animals were housed in a collective pen (12 x 4 m) on wheat-straw bedding and had free access to fresh water, minerals and salt blocks. The straw intake was not recorded or taken into consideration in feed intake data, but the behavior was monitored daily to ensure that ewes didn’t eat straw.

Two experimental diets were used during pregnancy: a) one based on dehydrated chopped oat hay (OH; 9.6% CP, 65.6% NDF, 4.9% ADL, DM basis; Table 1) fed ad libitum plus 350 g/d of whole corn grains and 140 g/d of soybean meal; and b) dehydrated chopped alfalfa hay of medium quality (AAM; 14.8% CP, 53.3% NDF, 8% ADL, DM basis; Table 1) fed ad libitum plus 440 g/d of whole corn grains. The soybean meal of the diet OH was used to compensate the low CP of the oat hay compared to the alfalfa hay (Table 1).

During early lactation both groups were fed the same diet, made by alfalfa dehydrated hay, the same used during pregnancy in the AAM group, fed ad libitum plus 440 g/d of whole corn grain + 135 g/d of soybean meal, DM basis; the latter was used to adapt the lactation diet to the lactation requirements of the ewes. The forage in both diets and in the two periods were fed ad libitum, while the concentrate was fed at fixed doses. Daily concentrate and forage feeding was divided into two equal meals at 08:00 and 16:00 h. The two groups of ewes, which used different diets during pregnancy but the same diet during the lactation, will be called, for the lactation stage, OHp and AAMp, to highlight their different diet during pregnancy.

2.2.2 Samplings and measurements

Late pregnancy

Dry matter intake (DMI) of forages was recorded daily and individually using the Biocontrol AS (Norway) mangers, which allow the individual measurement of the intake of each animal, identified by a specific RFID. The concentrates were supplied individually at milking and in manual individual mangers. The BW, BCS and BHB were recorded weekly and at the exact day at parturition. Feeds and refusals of forages were weighed daily for each group. Concentrates were always completely eaten. Feed samples for chemical analysis were taken weekly.

Early Lactation

DMI was recorded as described for the pregnancy period. Milk yield was recorded individually during the two daily milkings. Milk samples for milk quality analysis were taken, individually and separately for the two daily milkings, weekly. The BW was recorded daily by an automatic precision scale. The BCS (1-5 scale) was recorded by three operators and the average value among the three measurements was considered, while the BHB was obtained by using the blood ketone monitoring system Freestyle Optium Neo Monitor (Abbot Diabetis Care Ltd, UK) and the “FreeStyle optium beta-ketone (Abbot Diabetis Care Ltd, UK) strips and it was recorded weekly up to the 30 days in lactation. Feed and refusals were weighed daily for each group. Feed samples for chemical analysis were taken weekly.

2.2.3 Chemical analysis

The DM of feeds and refusals was determined weekly by drying the samples at 60 °C for 24 h. The dried feed samples were ground in a millet with a hammer mill using a sieve diameter of 1 mm. The ground samples of feeds and refusals were analyzed in an Ankom (NY, USA) fiber analyzer for aNDFom (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), following Van Soest et al. (1991). The NDF was analyzed by using heat stable α-amylase (Van Soest et al., 1991). Fiber fractions are reported on an ash-free basis. Crude protein (CP) was analyzed measuring total nitrogen with the Kjeldahl method and CP was calculated as total N x 6.25 (AOAC, 2004). Ashes were assessed with a muffle at 550 °C.

Methodology to calculate energy balance in late pregnancy and lactation

The energy balance during the pregnancy was calculated by using the equations to estimate nutrient supply and energy requirements for maintenance, late gestation and lactation proposed by the CNCPS-S method (Cannas et al., 2004), by using as inputs the data collected in the trial.

$$EB = MEI - (MEm + MEl + MEpreg)$$

where EB is ME balance, Mcal/d; MEI is ME intake, Mcal/d; MEm is ME required for maintenance, Mcal/d; MEl is ME required for milk production, Mcal/d; and MEpreg is ME required for pregnancy, Mcal/d.

2.2.4 Statistical analysis

Data for DMI, NDF intake, BW, BCS, BHB, estimated energy balance, milk yield and milk quality were analyzed statistically in a factorial design with feeding treatment, time and their interactions as fixed factors and animals as random factor, using the Mixed model of R software (RStudio for Windows, version 4.4.0).

The following linear mixed model has been adopted:

$$Yij = \mu + D + T + D*T + ID + e$$

Where:

Yij = Observations of the variables corresponding to the repetition j under the treatment of order i;

μ = Overall average of observations;

D = Fixed effect of the Treatment;

T = Fixed effect of the period or sampling;

D*T = Interaction between fixed effects of treatment and period;

ID = random effect of the animal;

e = Random error.

For a significant F-test (P<0.05), pair-wise comparisons between least square means of treatments were performed according to Tukey's test. The P-values are shown in tables as: *** (P<0.001), ** (P<0.01), * (P<0.05), (.) (tendency to significance, 0.05<P<0.10) or NS (no significance, P>0.10).

2.3 RESULTS

2.3.1 Voluntary feed intake in late pregnancy

Voluntary DMI (VDMI) and voluntary intake of hay decreased from day 121 in pregnancy up to lambing day, with a consistent drop during the last week before parturition (Figure 1). VDMI and voluntary intake of hay decreased more markedly in the OH group in respect to the AAM group. Considering the whole period, VDMI was higher in the AAM group compared to the OH Group (OH=1.161 vs. AAM=1.431 kg/d, P<0.01) as well as he voluntary intake of hay, which resulted higher in the AAM group compared to the OH group (OH=0.702 vs. AAM=1.023 kg/d, P<0.01; Table 2). The difference between the two groups in terms of hay eaten per day was considerable: on average the AAM group consumed +0.300 kg/d more hay than the OH group. The highest decrease in VDMI for the AAM group occurred at -6 DIP, while for the OH group occurred at -3 DIP (Figure 1).

Considering the VDMI expressed as percentage of body weight (VDMI%BW), the AAM group had a markedly higher VDMI%BW compared to the OH group (OH=2.03 vs. AAM=2.49, P<0.01; Figure 2) and this difference was maintained for the whole pregnancy, except for its last week, during which the VDMI%BW drop consistently in both groups, reaching the minimum of 1.34 DMI%BW for the OH group and 1.55 VDMI%BW for the AAM group. A similar pattern was observed for the level of intake of forages (as % of BW).

2.3.2 Intake of NDF and level of intake of NDF during late pregnancy

During the last month of pregnancy, the intake of NDF (NDFI) was higher in the AAM group compared to the OH group (OH=0.422 vs. AAM=0.588 kg/d, P<0.01; Table 2 and Figure 1). NDFI decreased from day 121 in pregnancy up to lambing day. However, the AAM group had a more constant NDFI during the whole period compared to the OH group (Figure 1). On average the AAM group was able to eat 0.166 kg/d more of NDF compared to the OH group (Table 2).

The same pattern resulted when NDFI was expressed as percentage of body weight (NDFI%BW): the AAM group had an higher NDFI%BW compared to the OH group (OH=0.73 vs. AAM=1.02 %BW, P<0.01; Table 2 and Figure 2) during the last month of pregnancy. The drop in the NDFI and NDFI%BW resulted for the AAM group at day -5 to lambing, and for the OH group at day -3 to lambing. Considering the whole period, the AAM group had an average NDFI%BW of 1.02 % of BW, while the OH group resulted in a lower NDFI%BW (0.73% of BW).

2.3.3 Body weight, body condition score and BHB in late pregnancy

No differences were observed about body weight (BW), even though the BW of AAM group was numerically higher then OH group (OH=58.7 vs. AAM=60.1 kg, NS; Table 3). However, there was a time effect for BW, considering that all the animals increased its BW approached lambing.

The body condition score (BCS) was also no different between the two groups (OH=2.88 vs. AAM=3.00, NS; Table 3).

The BHB during last month of pregnancy was affected by the diet (OH=0.54 vs. AAM=0.68, $P<0.05$; Table 3), resulting higher for the AAM group compared to the OH group.

2.3.4 Estimated energy balance in late pregnancy

The estimated energy balance (eEB) during the last 27 days in pregnancy resulted less negative for the AAM group compared to OH group (OH= -0.120 vs. AAM= -0.520 Mcal/d, $P<0.001$; Table 3, Figure 4). This difference was consistent during the whole period. The AAM group reached negative values of the eEB solely from in the last week of pregnancy, while the OH group entered in a negative energy balance after the first week of experimental treatment; consequently, the OH group remained in a negative energy balance for almost 20 days, while the AAM group for only the last 6 days.

2.3.5 Voluntary feed intake in early lactation

Voluntary DMI and voluntary intake of hay increased from day 1 after lambing up to day 28 in milk (DIM) (Table 4). The increase in VDMI and voluntary intake of hay was gradual and constant during the first 28 DIM, reaching the peak of intake at DIM 27 for the OHp group (fed OH during pregnancy) and DIM 28 for the AAMp group (fed AAM during pregnancy) (Figure 1). Considering the whole transition period, VDMI was numerically higher in the AAMp group compared to the OHp Group (OHp=1.873 vs. AAMp=1.992 kg/d, NS; Figure 1). The voluntary intake of hay resulted also numerically higher in the AAMp group compared to the OHp group (OHp =1.336 vs. AAMp=1.438 kg/d, NS).

Considering the VDMI expressed as percentage of body weight, the AAMp group had a numerically higher VDMI%BW compared to the OHp group (OHp=3.78 vs. AAMp=3.85 %BW, NS; Table 4 and Figure 2). The maximum values were achieved at the end of the lactation period considered (OHp=4.52% vs. AAMp=4.57% of BW, NS; Table 4 and Figure 2).

2.3.6 Intake of NDF and NDF related to body weight in early lactation

The intake of NDF during early lactation was numerically higher in the AAMp group compared to the OHp group (OHp=0.768 vs. AAMp=0.824 kg/d, NS; Table 4 and Figure 2). NDFI increased constantly from the 1st DIM until the 28th DIM. No differences resulted for the NDFI%BW, even if the AAM group had a numerically higher NDFI%BW compared to the OH group (OHp=1.55 vs. AAMp=1.59 %BW, NS; Table 4 and Figure 2) during the first month of lactation. Going from late pregnancy to early lactation, the NDFI%BW increase almost twofold, passing from 0.7-1.0 % of BW during pregnancy to 1.9-1.95 % of BW during lactation.

Body weight, body condition score and BHB in early lactation

The BW of AAMp group was numerically higher than that of the OHp group (OHp=50.1 vs. AAMp=51.6 kg, NS; Table 5). However, when considering the interaction of the diet with the time (D x T), significant differences has been observed from the 1st DIM to the 28th DIM (P<0.001). In the D x T interaction considered, the AAMp group had a higher BW compared to the OHp group.

Conversely the BCS during lactation was not affected from the diet supplied during pregnancy (OHp=2.88 vs. AAMp=3.00, NS; Table 5).

The BHB during the first month of lactation showed a tendency to be affected by the diet supplied during pregnancy (OHp=0.64 vs. AAMp=0.80, P=0.07; Table 5), resulting in a higher BHB for the AAMp group compared to the OHp group.

2.3.7 Estimated energy balance in early lactation

The estimated energy balance during the first 28 days in milk was not affected by the diet supplied during pregnancy (OHp= -0.205 vs. AAMp= -0.260 Mcal/d, NS; Table 5). The estimated energy balance became positive at 25-26 DIM in both groups.

2.3.8 Milk yield and milk composition

Milk yield (MY) during the first 28 DIM was not affected by the diet supplied during pregnancy: on average MY was 2.345 for the OHp group and 2.477 kg/d for the AAMp group (NS; Table 4). However, the AAMp group showed a numerically higher MY compared to the OHp group from the 3rd DIM to the 28th DIM. Only at DIM 1, 2 and 3 the OHp group had a numerically higher MY than the AAMp group. Considering the whole period, from the 1st DIM to the 28th DIM the difference in MY between the two groups was on average of +0.130 kg/d in favor of AAMp group.

Regarding the milk composition, milk fat percentage was affected by the diet supplied during the pregnancy period (OHp=5.08 vs. AAMp=5.47 %, P<0.01; Table 6). Considering each sampling date, the AAMp group had a higher milk fat percentage compared to the OHp group, the highest values of milk fat percentage have been registered at W1 in both groups (OHp=5.39 vs. AAMp=5.76 %, $P<0.01$; Table 6). Milk fat yield in grams per day was also affected by the diet supplied during pregnancy, showing higher levels in AAMp group compared to the OHp group, (on average OHp=131 vs. AAMp=144 g/d, $P<0.05$; Table). The highest levels of milk fat yield were reached at W4 (OHp=141 vs. AAMp=146 g/d, $P<0.05$; Table 6).

Milk protein percentage was not affected by the diet supplied during the pregnancy period (OHp=4.91 vs. AAMp=4.83 %, NS; Table 6). The highest values of milk protein percentage were registered at W1 in both groups (OHp=5.31 vs. AAMp=5.20 %, NS; Table 6). Milk protein yield in grams per day was also not affected by the diet supplied during pregnancy, showing similar levels in both groups for all the sampling dates, on average (OHp=126 vs. AAMp=127 g/d, NS; Table 8). The highest levels of milk protein yield were reached at W4 (OHp=132 vs. AAMp=131 g/d, NS; Table 6).

The fat vs. protein ratio (F/P) was influenced by the diet supplied during pregnancy; more precisely the AAMp group showed higher values of F/P compared to the OHp group, on average (OHp=1.04 vs. AAMp=1.14, $P<0.01$; Table 6). This pattern was observed for all the sampling dates.

Lactose percentage, somatic cell count, casein percentage as well as casein yield and urea were not affected by the diet supplied during pregnancy, while milk pH was affected, being higher in the AAMp group (OHp=6.76 vs. AAMp=6.81, $P<0.01$; Table 6).

2.4 DISCUSSION

2.4.1 Effects of forage quality on dairy ewes performances

2.4.1.1 The voluntary intake of ewes in late pregnancy

The VDMI of late gestating ewes was clearly affected by the forage quality. Ewes showed a higher intake for the forage of highest quality, the dehydrated chopped alfalfa hay, compared to the dehydrated chopped oat hay (Figure 1). The highest VDMI of the AAM group was able to cover, for almost the whole period and except for the last week, the energy requirements of pregnancy. On the contrary, the OH group, which had a lower VDMI, was not able to ingest the proper amount of forage to cover completely their energy requirements of pregnancy (Figure 4). This difference confirmed the well-known fact that high quality forages, with lower NDF and likely higher DM digestibility, which have higher VDMI compared to poorer quality forages. Indeed, in our study the oat hay was richer in NDF than the AAM (AAM=53.3 vs. OH=65.7% NDF). Despite the forage type was different (dehydrated forage vs. haylage), our results are in concordance with those of Helander et al., (2014a), who showed that the VDMI of ewes during late pregnancy was higher when the haylage had lower NDF (haylage cut in two different years, with 58.0% and 48.3 % of NDF in the two years). The difference in VDMI of our experiment might be explained largely by the bulkiness of the OH diet, which seemed to have caused a fill effect in the rumen. In fact, the ewes of the OH group were able to eat 0.6-0.7 kg/d of hay (DM basis) compared to 1.0 kg/d (DM basis) of the AAM group. In a similar study conducted by Jalali et al. (2012a), the intake of hay decreased when supplying diet with grass silage harvested at different maturity to pregnant sheep (Early Harvested (EH)= 2.73; Medium Harvested (MH)= 2.40; Late Harvested (LH)= 2.00 kg/d), this decrease in intake was caused by the increasing level of NDF percentage of the silage (EH= 44.9%, MH= 57.8%; LH= 63.4 %NDF). These findings are also in line with the theory proposed firstly by Waldo (1986), and more recently confirmed by Ingvartsen and Anderson (2000) and Mertens (2002b), which explained that VDMI is limited by the energy density of the diet when supplying high energy diets or might be limited by the NDF of the diet, in that case the bulkiness of the forage would limit the intake causing a fill effect at rumen level.

Interestingly, in our experiment both groups maintained their intake constant during the whole period, with an oscillatory pattern except for the last week before parturition, in which the decrease in intake was linear and constant (Figure 1). It seemed that in both groups the intake on the last week was not governed by a diet effect, such as it was in the previous period. In fact, during the last week of pregnancy both AAM and OH groups decreased simultaneously the VDMI, with a consistent drop on the last 5 to 3 days before parturition. Similar results of VDMI patterns were observed in dairy cattle during the last week of pregnancy (Murphy, 1999). This aspect reflected more than a diet effect a physiological and hormonal status that in that moment had a higher impact on the behavior of the animal. It could be possible that in the last week, first, the higher compression of the fetus against the rumen and secondly the changes in hormonal status may interfere on the appetite and stress of the animal (Forbes, 1969; Ingvartsen et al., 1999).

Considering the DMI as proportion of BW, we observed that the values obtained for the AAM group (2.49 % of BW) were not far different from those reported from by Macedo Junior et al. (2012) from late gestating ewes of similar body weight. A major positive difference was observed with the values reported by Olsen (2016) for meat sheep of higher body weight. Helander et al, (2014a) reported higher values of DMI%BW in Experiment 1 when feeding during late pregnancy Unchopped Silage (US) vs Chopped Silage (CS) vs Chopped Silage plus concentrates (CM), the percentage of NDF of the silage used was 58.0 %. Results showed differences in DMI%BW when compared to our data (OH=2.03; AAM=2.49 vs US=2.86; CS=2.86; CM=2.77 DMI%BW).

Regarding the NDF intake as proportion of the BW, group OH reached only 0.72% of BW, which is far from the value of 1.0% of BW reported by Cannas et al. (2016), based on a literature review, for pregnant ewes, while the AAM group had a NDFI of 1.02 % of BW (Table 2), similar to that indicated by Cannas et al., (2016). Probably the OH group was not able to achieve the threshold of 1.0 % of BW because the bulkiness of the oat hay limited the voluntary intake. Comparing our results with those of similar studies, it appears that the level of NDFI (% of BW) was similar among the studies considered, with little differences due to the breed and the body weight of the animals but large differences caused by the forage quality. Nielsen (2016) reported a NDFI of 1.03 of BW, a value that varied very little in pregnant sheep of 95.6 kg of body weight when fed different quality of forages and concentrates. Macedo Junior et al. (2012) found higher values of NDFI (% BW) in 130 d pregnant sheep compared to those of our study, carrying single and twins and fed restricted or unrestricted diets (restricted, 1 fetus= 66% NDF; unrestricted, 1 fetus=50% NDF; restricted, 2 fetuses=55% NDF; unrestricted, 2 fetuses=41% NDF). On average, in this publication the NDFI was 1.49 % of BW for the unrestricted vs. 1.76 % of BW for the restricted group, suggesting that in this study NDFI was cause an extreme rumen fill, underling the effect both of the litter size and the nutritional management. Jalali et al. (2012a) found values of NDFI of 1.13 (%BW) when feeding early harvested grass silage(NDF=44.9%; ME=11.7MJ/kgDM)., 1.2 % of BW when feeding medium harvested grass silage (NDF=57.8%; ME=10.8MJ/kgDM) and 1.33% of BW when feeding late harvested grass silage (NDF=63.8%; ME=9.3MJ/kgDM) in pregnant ewes of 85 kg of BW. Thus, it is evident that the ewes with the lowest quality of forage (late harvested grass silage) were trying to eat more to achieve higher quantity of energy from the forage source which being of poor nutritional quality had in turns a lower density of energy per kg of dry matter (ME=9.3 MJ/kg DM). Helander et al. (2012a) reported higher values of NDFI (% BW) in two consecutive years of experiment conducted in pregnant ewes (97 kg of BW): on average the NDFI was 1.4% of BW in the first year (58% of NDF) vs. 1.22 % of BW in the second year (NDF 48%). The difference might be explained considering the difference in NDF content of the haylage, with the worst one maximizing the rumen fill.

Thus, the level of NDFI of 1% of BW achieved in Sarda ewes with the AAM diet might be considered as close to the optimum for the feeding regimen considered (i.e. forage ad libitum and fixed concentrate supply),

2.4.1.2 The evolution of BW, BCS, and estimated energy balance during pregnancy

The BW during pregnancy resulted numerically higher in the AAM group, whose weight was +1.4 kg higher compared to the OH group. The higher BW might be explained by the highest VDMI of the group, which had more energy from the diet to be converted in body reserve. In fact, also the BCS of the AAM group was numerically higher than the OH group, with a difference of a quarter of point. In support of these findings, the estimated energy balance resulted less negative in the AAM group, with a difference between the two groups of -0.40 Mcal/d, suggesting that the AAM, due to its higher intake, had satisfied its energy requirements of pregnancy for almost the whole pregnancy stage considered, while the OH group was not able to cover its requirements.

2.4.1.3 The voluntary intake of ewes in early lactation

The data on VDMI during lactation suggested that the diet supplied during pregnancy did not affect the intake in early lactation. However, the AAMp group had a numerically higher DMI in the first days after parturition compared to the OHp group. This pattern has been maintained until the 12th days in milk, when the OHp group had similar values of DMI than to the AAMp group (AAMp=1.831 vs. OHp=1.824 kg/d). Conversely, Helander et al. (2014a) observed higher values of DMI when chopped silage (CS) or chopped silage plus concentrate (CM) were fed to early lactating ewes in respect to those fed with unchopped silage (US), (CS=3.87; CM=4.36; US=3.73 kg/d), respectively. In regard to the NDFI, we observed similar values in respect to those reported by Hubner et al. (2007), in which early lactating ewes were fed at three different NDF levels of the diet (34, 43 and 52% of DM). These findings suggest that ewes of similar body weight had approximately the same intake of NDF, when comparing similar quality of the diet, moreover when comparing our results with those of Hubner et al. (2007) for the same NDF level of the diet, we observed also similar values of DMI and NDFI (%BW) compared with Hubner et al. (2007). Confronting our data with the reference values proposed by Cannas et al. (2016), we observed a little discrepancy for ewes producing 2.5 kg/d of milk (corrected for 6.5% fat and 5.8% protein) of 45 kg of BW. The reference values of Cannas et al (2016) suggest 5.6% of DMI (% of BW) for a level of 2.10 NDFI (% of BW), which is the threshold at which the NDF didn't restrict the DMI due to rumen fill. In our study, we observed an average DMI of 3.80% of BW (Table 4) with a NDFI of 1.59% of BW. These results suggest that during the early lactation period the animal took at least 28 days to reach the optimal levels of voluntary intake, both for DM and NDF. This agrees with the DMI model of Dove, reported by the NRC (2007), which indicates that sheep reach maximum intake one month after lambing. Values of NDFI like those proposed by Cannas et al. (2016) were reached solely approaching the 28th days in milk. Therefore, from the results reported, the quality of the forage used and the NDF of the diet did not limit the intake of the ewes in the first month of lactation, but rather allowed animal to increase their intake day by day, in concordance with the remodulation of the rumen volume and the increase in energy requirements of lactation after lambing.

2.4.2 The evolution of BW, BCS, BHB and estimated energy Balance during early lactation

The BW during early lactation was constant for both groups, with little lower values for the OHp group compared to the AAMp group. . Likely, this happened because the AAMp group eat more during pregnancy and early lactation, limiting body reserve losses. However, the there were no differences in BW evolution during lactation between the two groups. Because of this, in fact, also the BCS has not changed drastically during the first 28 days. No evidence of subclinical ketosis associated to the diets was observed, as indirectly indicated by the BHB, which in both groups was, indeed, slightly above or below the threshold of subketosis of 0.8 mmol/l (Lacetera et al., 2001, 2002). A similar pattern for BHB was observed by Molina et al. (2001), who reported the peak of BHB in proximity to the lambing day. The estimated energy balance was negative in both groups, as expected in animals in early lactation. The eEB started to raise and became positive at 20th DIM, which corresponds to the DIM at which the ewes reached 2 kg/d of DMI.

2.4.3 The effect of forage quality on milk yield and milk composition

Milk yield was not influenced by the diet supplied during pregnancy. However, across the first 28th DIM, the AAMp group showed a consistent numerically higher milk yield compared to the OHp group, on average (2.477 vs. 2.345 kg/d), even if during the lactation they were fed the same diet. An explanation of this pattern might come from the behaviour of DMI, as previously discussed. In fact, the AAMp group had a higher DMI after lambing, which may have led to a higher MY. Likely, the better quality of the forage used in pregnancy for the AAM group induced a better mammary development, and thus higher numerical milk production and subsequent higher DMI. However, since also the OHp group showed high value of MY (2.345 kg/d), we hypothesize that the feed regimen at which the pregnancy OH group was fed during early lactation may have compensated, at least partially, the undernutrition at which the OH ewes were subjected during pregnancy. Considering that the diet supplied was based of forages fed al libitum and a fixed, fairly limited supplementation of concentrate, the productions obtained were very high if compared to what is reported in the bibliography for sheep of the same breed (Cannas et al., 2002; Cannas, 2004; Molle et al, 2014). Probably, not only the quality of the forage supplied but also the nutritional management allowed the animals to exploit their genetic potential. In fact, if we compare our results with similar studies, the values of milk production were higher than those reported by Hubner et al. (2007) and Molina et al. (2001).

2.5 CONCLUSIONS

The utilization of forages of high quality (dehydrated chopped alfalfa hay) during the late pregnancy period had a positive impact on the VDMI, hay intake, NDFI and the eEB of the ewes when compared to a forage of low quality (dehydrated chopped oat hay). The AAM diet allowed the animals to have higher DMI and higher intake of hay, which in turns permitted the animals to obtain more energy from the fibrous sources of the diet, since the quantity of concentrate was fixed in both groups. Consequently, the AAM arrived at the lambing with a less negative energy balance compared to the OH group. Our findings confirmed that the reference value of NDFI of 1.0-1.1 % of BW proposed by Cannas et al. (2016) is the optimal value to avoid undefeeding or overstreching of rumen capacity. From the results obtained, there was not a clear negative effect of the diet supplied during late pregnancy on the subsequent early lactation performances in terms of DMI, NDFI and milk yield, when the ewes were fed during early lactation the same diet of high quality. Thus, it seems that the utilization of forages of high quality during early lactation may partially compensate the undernutrition at which ewes were exposed during the last month of pregnancy, due to the utilization of low quality OH forage. In consideration of what we observed in this study, the utilization of high-quality forages must be considered as a pivotal point nutritional management of the transition from pregnancy to lactation. However, further information is necessary to compare high- and low-quality forages during early and subsequent periods of lactation.

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2.7 TABLES AND FIGURES

Table 1. Chemical composition of forages and concentrates supplied during late pregnancy and early lactation

	Forage			**Concentrate**	
	Pregnancy		**Lactation**		
	Oat hay (OH)	**Alfalfa hay medium (AAM)**	**Alfalfa hay medium (AAM)**	**Whole corn grains**	**Soybean meal**
DM*, %	88.6	89.0	89.0	88.0	90.0
CP, %DM	9.6	14.8	14.8	9.8	49.9
NDF, %DM	65.7	53.3	53.3	10.0	14.9
ADF, %DM	38.5	43.7	43.7	-	-
ADL, %DM	4.9	9.8	9.8	0.2	0.3
Starch. %DM	-	-	-	73.8	2.1
NFC, %DM	16.6	22.7	22.7	75.8	28.9
Ash, %DM	8.8	9.0	9.0	1.6	7.2
EE, %DM	2.2	1.8	1.8	4.3	1.6
Mineral composition**					

DM*=dry matter

Mineral composition:** Ca = 146 g, P = 30 g, Mg = 38 g, Na = 130 g, I = 50 mg, Mn = 1.800 mg, Se = 8,3 mg, Zn = 2.880 mg, Vitamin A = 500.000 U.I., Vitamin D3 = 50.000 U.I., Vitamin E = 1244 U.I., Vitamin B1 = 15 mg, Vitamin B2 = 18 mg, Vitamin B6 = 9 mg, Vitamin B12 = 52,7 mg

Table 2. Evolution of dry matter intake (DMI, kg/d)), dry matter intake expressed as percentage of body weight (DMI, %BW), hay dry matter intake (hay DMI, kg/d), NDF intake (NDFI, kg/d) and NDF intake expressed as percentage of body weight (NDFI, %BW) of Sarda dairy ewes during the last 27 days of pregnancy, fed dehydrated chopped oat hay (OH) or dehydrated chopped alfalfa hay (AAM) plus fixed doses of concentrates.

	Diet	**Sampling intervals - Pregnancy**									**Mean**	**SE**[a]	**P-value**		
		-T9**	**-T8**	**-T7**	**-T6**	**-T5**	**-T4**	**-T3**	**-T2**	**-T1**			**D**[b]	**T**[b]	**D*T**[b]
DMI, kg/d	OH	1.310	1.220	1.079	1.084	1.118	1.202	1.222	1.191	1.029	1.161	0.06	**	***	***
	AAM	1.383	1.469	1.446	1.495	1.425	1.518	1.540	1.483	1.121	1.431	0.06			
DMI, %BW	OH	2.36	2.19	1.93	1.95	1.92	2.08	2.10	2.01	1.73	2.03	0.06	*	***	***
	AAM	2.57	2.69	2.56	2.63	2.45	2.60	2.59	2.47	1.86	2.49	0.06			
Hay DMI, kg/d	OH	0.851	0.773	0.630	0.613	0.635	0.710	0.736	0.744	0.627	0.702	0.06	**	***	***
	AAM	0.965	1.041	1.028	1.071	1.023	1.095	1.124	1.080	0.785	1.023	0.06			
NDFI, kg/d	OH	0.501	0.459	0.383	0.376	0.388	0.430	0.433	0.443	0.376	0.422	0.03	**	***	***
	AAM	0.558	0.599	0.592	0.615	0.587	0.627	0.642	0.618	0.453	0.588	0.03			
NDFI, %BW	OH	0.89	0.81	0.68	0.67	0.66	0.74	0.75	0.74	0.62	0.73	0.07	**	***	***
	AAM	1.04	1.09	1.05	1.08	1.01	1.07	1.07	1.02	0.74	1.02	0.07			

[a] SE=standard error of the mean

[b] T=time, D=diet, D*T=interaction Diet x Time

*P<0.05, **P<0.01, ***P<0.001, NS=not significant

**T= each T include 3 experimental days of data

Table 3. Evolution of body weight (BW, kg), body condition score (BCS, 1-5), beta-hydroxybutyrate (BHB, mmol/L) and estimated energy balance (eEB, Mcal/d) of Sarda dairy ewes during the last 27 days of pregnancy, fed dehydrated chopped oat hay (OH) or dehydrated chopped alfalfa hay (AAM) plus fixed doses of concentrates.

	Diet	**Sampling intervals – Pregnancy**				**Mean**	**SE**[a]	**P-value**		
		--T9**	**-T6**	**-T3**	**-T1**			**D**[b]	**T**[b]	**D*T**[b]
BW, kg	OH	56.6	58.5	59.5	60.0	58.7	2.19	NS	***	NS
	AAM	57.5	59.4	61.1	62.6	60.1	2.19			
BCS (1-5)	OH	2.91	2.96	2.86	2.81	2.88	0.08	NS	*	NS
	AAM	3.00	3.07	2.99	2.94	3.00	0.08			
BHB (mmol/L)	OH	0.46	0.50	0.54	0.64	0.54	0.04	*	***	NS
	AAM	0.63	0.60	0.60	0.89	0.68	0.04			
eEB, Mcal/d	OH	0.22	-0.57	-0.63	-1.45	-0.52	0.05	***	***	NS
	AAM	0.24	0.16	-0.11	-1.36	-0.12	0.05			

[a] SE=standard error of the mean
[b] T=time, D=diet, D*T=interaction Diet x Time
*P<0.05, **P<0.01, ***P<0.001, NS=not significant
**T= each T include 3 experimental days of data

Table 4. Evolution of dry matter intake (DMI, kg/d)), dry matter intake expressed as percentage of body weight (DMI, %BW), hay dry matter intake (hay DMI, kg/d), NDF intake (NDFI, kg/d) and NDF intake expressed as percentage of body weight (NDFI, %BW) and milk yield (MY, kg/d)) of Sarda dairy ewes during the first 28 days in milk (DIM), fed during pregnancy the OH and AAM diets, and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate**.**

	Diet during pregnancy	**Sampling intervals - LACTATION**														**Mean**	**SE^a**	**P-value**		
		T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**	**T13**	**T14**			**D^b**	**T^b**	**D*T^b**
DMI, kg/d	OH	1.276	1.555	1.533	1.678	1.777	1.824	1.912	1.953	1.990	2.051	2.075	2.116	2.235	2.253	1.873	0.08	NS	***	NS
	AAM	1.517	1.706	1.632	1.837	1.857	1.831	2.012	2.101	2.087	2.200	2.198	2.223	2.320	2.372	1.992	0.08			
DMI, %BW	OH	2.33	3.13	3.12	3.44	3.60	3.74	3.88	3.98	4.06	4.15	4.22	4.24	4.50	4.52	3.78	0.16	NS	***	NS
	AAM	2.69	3.25	3.15	3.59	3.60	3.63	3.95	4.07	4.08	4.29	4.29	4.28	4.46	4.57	3.85	0.15			
Hay DMI, kg/d	OH	0.874	1.081	1.037	1.140	1.231	1.284	1.366	1.395	1.425	1.438	1.503	1.544	1.663	1.681	1.336	0.02	NS	***	NS
	AAM	1.011	1.177	1.120	1.296	1.200	1.285	1.442	1.538	1.515	1.628	1.626	1.651	1.748	1.800	1.438	0.02			
NDFI, kg/d	OH	0.508	0.626	0.604	0.664	0.713	0.740	0.785	0.801	0.818	0.850	0.861	0.882	0.946	0.955	0.768	0.04	NS	***	NS
	AAM	0.591	0.682	0.650	0.747	0.751	0.742	0.828	0.878	0.867	0.927	0.926	0.940	0.991	1.019	0.824	0.04			
NDFI, %BW	OH	0.92	1.26	1.23	1.36	1.44	1.51	1.59	1.63	1.67	1.72	1.74	1.76	1.90	1.91	1.55	0.08	NS	***	NS
	AAM	1.04	1.29	1.24	1.45	1.44	1.46	1.62	1.69	1.68	1.80	1.80	1.80	1.89	1.95	1.59	0.07			
MY, kg/d	OH	1.341	1.606	2.100	2.291	2.309	2.426	2.435	2.476	2.535	2.552	2.592	2.716	2.703	2.745	2.345	0.04	NS	***	NS
	AAM	1.249	1.690	2.224	2.451	2.582	2.499	2.540	2.751	2.746	2.708	2.841	2.762	2.804	2.826	2.477	0.04			

[a]SE=standard error of the mean
[b]T=time, D=diet, D*T=interaction Diet x Time
*P<0.05, **P<0.01, ***P<0.001, NS=not significant
**T= each T include 2 experimental days of data

Tabella 5. Evolution of body weight (BW, kg), (BCS, 1-5), beta-hydroxybutyrate (BHB, mmol/L) and estimated energy balance (eEB, Mcal/d) of Sarda dairy ewes during the first 28 days in milk (DIM), fed during pregnancy dehydrated chopped oat hay (OH) and dehydrated chopped alfalfa hay (AAM) plus fixed doses of concentrates, and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate**.**

	Diet during pregnancy	**Sampling intervals - LACTATION**				**Mean**	**SE**[a]	**P-value**		
		T3**	**T6**	**T9**	**T12**			**D**[b]	**T**[b]	**D*T**[b]
BW, kg	OH	49.3	49.2	49.5	50.1	50.1	0.31	NS	***	***
	AAM	51.3	50.7	51.3	52.0	51.6	0.30			
BCS (1-5)	OH	2.77	2.71	2.71	2.79	2.74	0.05	NS	NS	NS
	AAM	2.79	2.75	2.79	2.77	2.77	0.05			
BHB, mmol/L	OH	0.56	0.70	0.70	0.62	0.64	0.04	0.07	NS	0.05
	AAM	0.90	0.88	0.72	0.70	0.80	0.04			
eEB, Mcal/d	OH	-0.77	-0.42	-0.03	-0.05	-0.205	0.05	NS	***	NS
	AAM	-0.86	-0.67	-0.27	-0.01	-0.260	0.05			

[a] SE=standard error of the mean

[b] T=time, D=diet, D*T=interaction Diet x Time

*P<0.05, **P<0.01, ***P<0.001, NS=not significant

**T= each T include 2 experimental days of data

Table 6. Chemical composition of milk yield during the first 28 days in milk (DIM) in sarda dairy ewes

	Diet during pregnancy	Sampling intervals - LACTATION				Mean	SE[a]	P-value		
		T3**	T6	T9	T12			D[b]	T[b]	D*T[b]
Fat, %	OH	5.39	5.14	4.75	5.02	5.08	0.10	**	*	NS
	AAM	5.76	5.59	5.28	5.28	5.47	0.09			
Fat, g/d	OH	129	129	125	141	131	4.69	0.05	NS	NS
	AAM	138	146	145	146	144	4.50			
Protein, %	OH	5.31	4.85	4.74	4.74	4.91	0.04	NS	***	NS
	AAM	5.20	4.70	4.69	4.74	4.83	0.04			
Protein, g/d	OH	126	121	125	132	126	3.85	NS	NS	NS
	AAM	124	123	129	131	127	3.70			
F/P	OH	1.02	1.07	1.00	1.06	1.04	0.02	**	NS	NS
	AAM	1.12	1.20	1.13	1.12	1.14	0.02			
Lactose, %	OH	5.12	5.16	5.17	5.17	5.16	0.02	NS	NS	NS
	AAM	5.06	5.16	5.15	5.19	5.14	0.02			
CCS x1000	OH	167.4	849.8	59.2	54.0	282.6	135	NS	NS	NS
	AAM	157.6	51.0	47.5	46.3	75.6	130			
Caseine, %	OH	3.91	3.59	3.53	3.55	3.64	0.03	NS	***	NS
	AAM	3.78	3.46	3.46	3.53	3.56	0.03			
Caseine, g/d	OH	92.9	90.0	92.9	99.1	93.7	2.93	NS	NS	NS
	AAM	90.5	91.0	95.6	98.1	93.8	2.81			
Urea, dL/ml	OH	48.7	42.0	42.5	44.7	44.5	1.22	NS	NS	NS
	AAM	45.1	40.7	47.8	47.4	45.2	1.17			
pH	OH	6.83	6.77	6.72	6.75	6.76	0.01	**	***	NS
	AAM	6.93	6.80	6.74	6.80	6.81	0.01			
Cryoscopy	OH	0.572	0.569	0.573	0.574	0.572	0.001	NS	**	NS
	AAM	0.566	0.570	0.575	0.579	0.572	0.001			
NaCl	OH	95.3	101.8	103.4	102.0	101	3.45	NS	NS	NS
	AAM	100.1	101.9	106.3	105.1	103	3.31			

[a] SE=standard error of the mean

[b] T=time, D=diet, D*T=interaction Diet x Time

*P<0.05, **P<0.01, ***P<0.001, NS=not significant

**T= each T include 2 experimental days of data

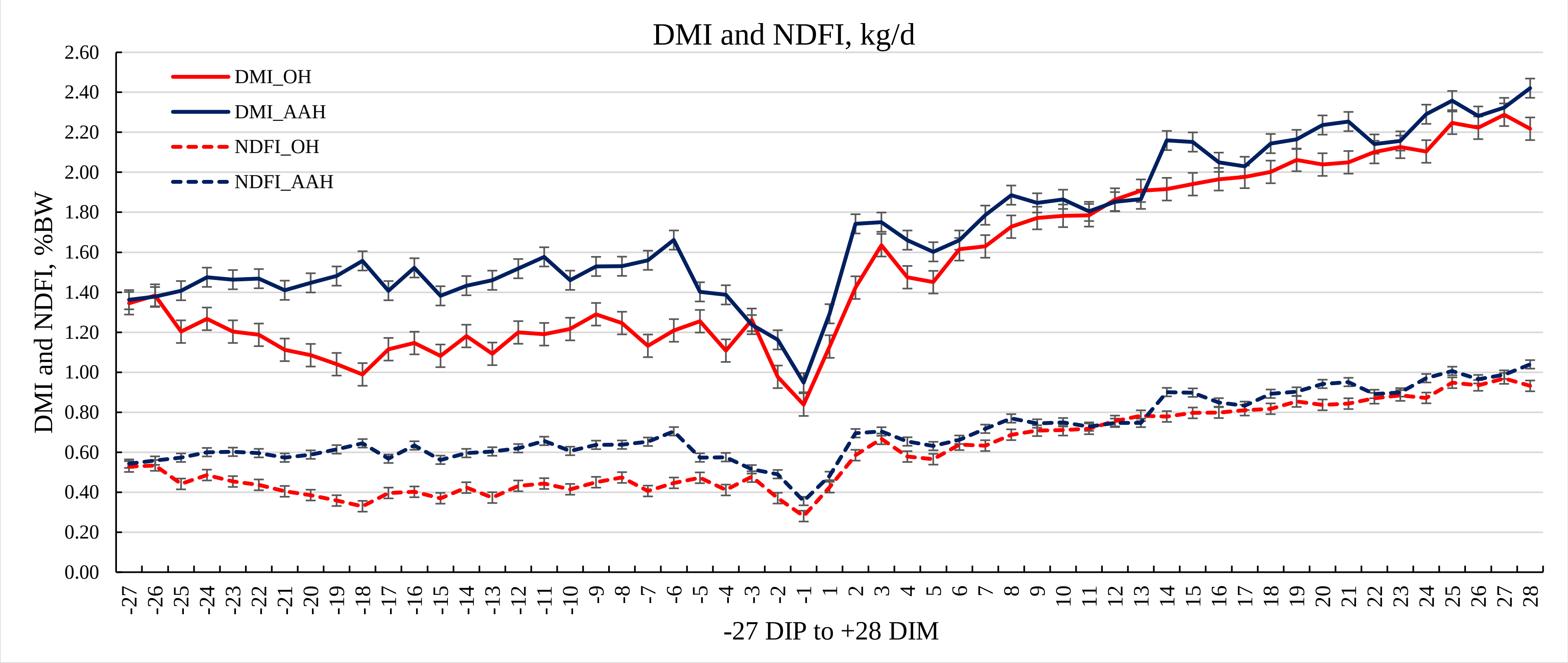

Figure 1. Evolution of DMI and NDFI in kg/d, in sarda dairy ewes during the transition period (-27 days in pregnancy to +28 days in milk) fed dehydrated chopped oat hay (OH) and dehydrated chopped alfalfa hay (AAM) during pregnancy and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate

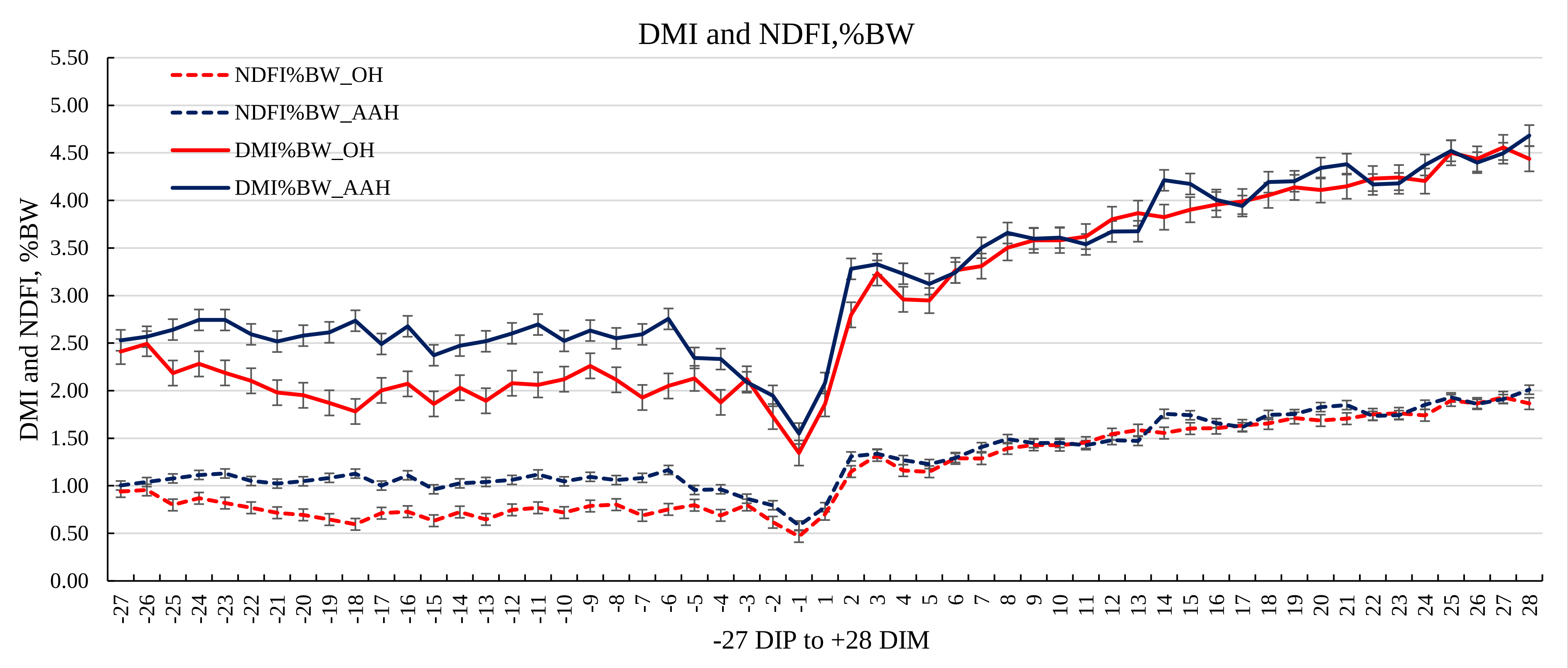

Figure 2. Evolution of DMI and NDFI as percentage of body weight (%BW), in sarda dairy ewes during the transition period (-27 days in pregnancy to +28 days in milk) fed dehydrated chopped oat hay (OH) and dehydrated chopped alfalfa hay (AAM) during pregnancy and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate.

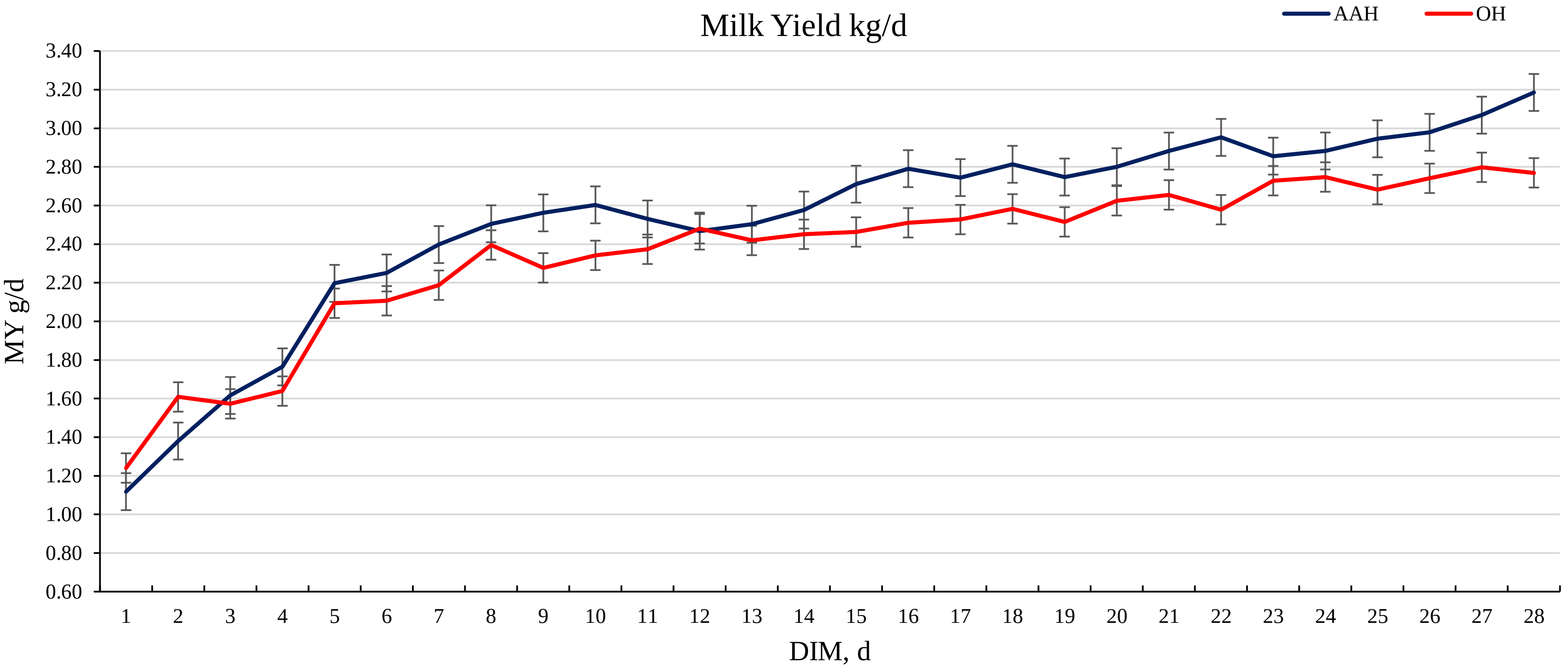

Figure 3. Evolution of Milk Yield in kg/d, in sarda dairy ewes during the first 28 days in milk (DIM) when fed dehydrated chopped oat hay (OH) and dehydrated chopped alfalfa hay (AAM) during pregnancy and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate.

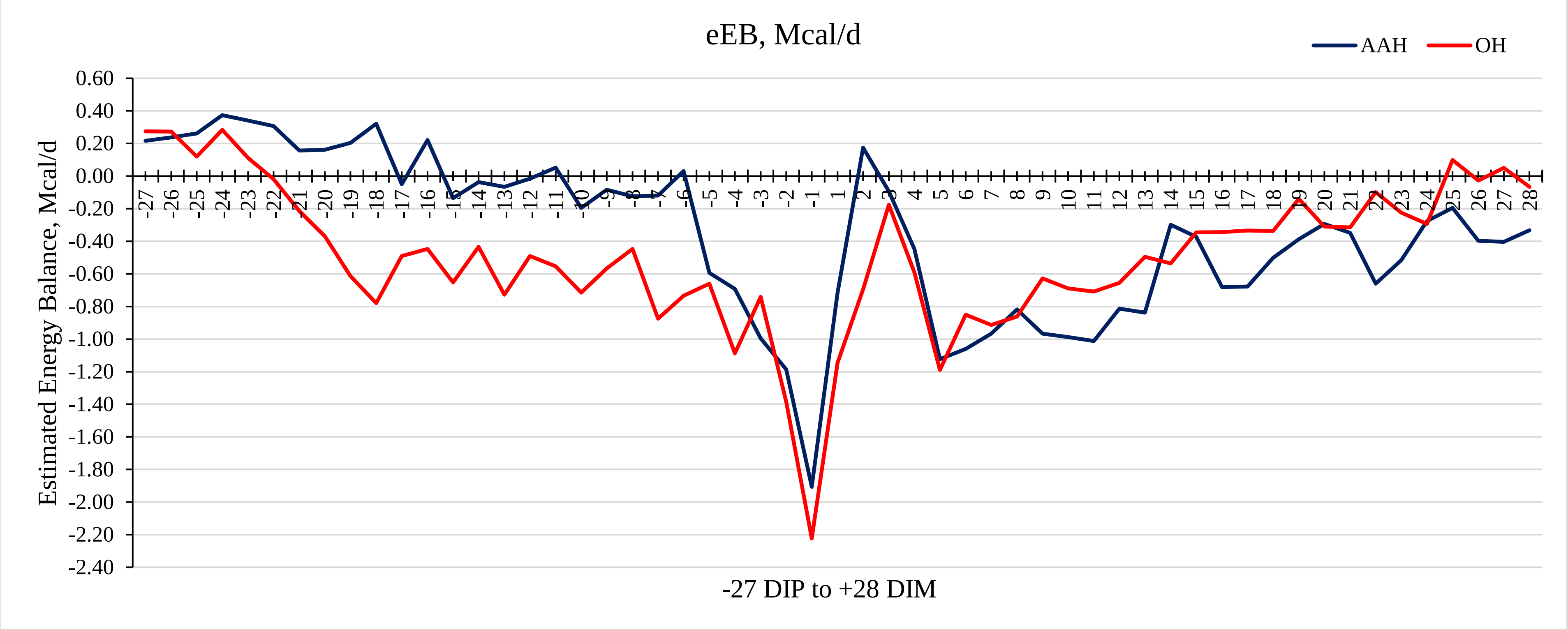

Figure 4. Evolution of estimated Energy Balance (eEB) in Mcal/d, in sarda dairy ewes during the transition period (-27 days in pregnancy to +28 days in milk) fed dehydrated chopped oat hay (OH) and dehydrated chopped alfalfa hay (AAM) during pregnancy and a common diet of dehydrated chopped alfalfa hay (AAM) during lactation, plus fixed doses of concentrate

3 CHAPTER 3

Effects of three different forages fed during mid lactation on intake and performances of Sarda dairy ewes

ABSTRACT

The importance of forage quality on the intake and milk production has been deeply explored in dairy cattle, while quantitative information is lacking in lactating dairy ewes. Previous studies have shown that the lactation persistency of dairy ewes in mid-lactation is negatively affected by the utilization of medium-high doses of sugars and starch, while is positively affected by the utilization of highly degradable fiber sources. For this reason, the choice of the appropriate forage sources is particularly important.

Thus, this study evaluated the productive effects of three different forage sources fed ad libitum. Twenty-one Sarda dairy ewes in the 5th month of lactation were divided into three groups balanced for DMI, milk yield (MY), body weight (BW) and BCS, mean±SD; DMI 2.22±0.05 kg/d MY 2.00±0.03 kg/d; BW 53.9±2.04 kg; BCS 2.98±0.08. One group received dehydrated chopped OH hay (OH; 7.3% CP, 63.9% NDF, 38.5% ADF, 4.78% ADL, DM basis) ad libitum and a supplementation of 176 g/d of whole corn grains and 360 g/d of soybean meal, DM basis. The second and third group received dehydrated chopped alfalfa hay of medium quality (AAM); 19.7% CP, 43.5% NDF, 43.8% ADF, 6.48% ADL, DM basis) and high quality (AAH) (23.5% CP, 39.0% NDF, 43.3% ADF, 5.8% ADL, DM basis) ad libitum, respectively, with a supplementation for both cases of 528 g of DM/d of whole corn grains. The experimental period lasted 21 d. A factorial design with feeding treatment, time and their interactions as fixed factors, animals as random effect was applied.

The diets affected the voluntary intake of hay, voluntary DMI (OH=1.289 vs AAM=2.085 vs AAH=2.733 kg/d, $p<0.001$), and NDF intake per day (OH=0.587 vs AAM=0.738 vs AAH=0.903 kg/d, $p<0.001$) and as % of BW. All were markedly reduced as forage quality decreased. Milk yield was also affected by the diets (OH=1.491 vs AAM=1.755 vs AAH=2.028 kg/d, $p<0.001$), being markedly reduced as forage quality decreased.

The quality of the diet significantly affected the chemical composition of milk, with the OH group having higher fat and protein percentages, but the AAH group had highest milk fat and protein yield, and thus highest quantities of milk components suitable for cheesemaking. Urea content in the milk was highest in the AAH group, probably because its highest intake of protein.

This study highlighted the importance of feeding high quality forage during the mid-lactation phase to maintain the persistency of lactation and improve milk quality parameters.

Key words: **Forage quality, mid lactation, forage, Voluntary DMI, NDF**

3.1 INTRODUCTION

The dairy industry plays a pivotal role in meeting the global demand for high-quality milk and dairy products. Within this industry, dairy sheep, specifically lactating ewes, contribute significantly to milk production (Pulina et al., 2018). The efficient management of ewes during mid-lactation is paramount to achieve optimal milk yields. Among the numerous factors influencing milk production in dairy sheep, the quality of forage has emerged as a critical determinant (Cannas, 2004). While the importance of forage quality on feed intake and milk production has been extensively studied in dairy cattle, a notable gap exists when it comes to its impact on lactating dairy ewes, particularly during the mid-lactation phase. Mid-lactation is a fundamental phase for dairy ewes, as it represents the peak of their lactation curve and is associated with the highest milk production (Pulina et al., 2007). In addition to the challenges posed by forage quality, the persistence of lactation in dairy sheep such as in dairy cattle is a critical factor that significantly influences overall milk production and farm profitability (Ekkers et al., 1998). Lactation persistency refers to the ability of ewes to maintain high milk yields over an extended period, beyond the initial peak of milk production. It is a key determinant of overall milk production efficiency and can be affected by various factors, including non-nutritional and nutritional (Cannas et al., 2007; Pulina et al., 2007). Understanding how forage quality affects milk production in this specific context is crucial for enhancing the overall efficiency and sustainability of dairy sheep operations. On this regard, mature forages could have negative impact on both feed intake and milk production (Oba and Allen, 1999; Cannas, 2004; Steinshamn, 2010). Mature forages, characterized by their high fiber and lignin content and low fiber rumen degradability, can pose challenges to lactating ewes by limiting nutrient availability and slowing down the digestion process, as showed by Mertens (1993;1994a) in cattle. This can hinder milk production and impact the overall performance of dairy ewes.

In dairy ewes, especially during mid lactation, several experiments have demonstrated that diets abundant in highly digestible fiber fron non-forage surces, such as those with elevated levels of beet pulps or soybean hulls, led to an increase in milk production persistency, while diets high in starch promoted fattening (Cannas et al., 1998; Bovera et al., 2004; Zenou and Miron, 2008; Cannas et al., 2013; Lunesu et al., 2021). On the contrary, the advantageous impact of highly digestible fiber during mid and late lactation has not been observed in gOHs. Instead, gOHs in these lactation stages exhibited a positive response to high-starch diets (Cannas et al., 2013; Lunesu et al., 2021). Considering this, the utilization of high quality forages, which have more digestible and fermentable fiber, might have a key role in stimulating milk production during mid and late lactation.

Furthermore, diets rich in feeds by-products rich in highly digestible fiber have a positive effect on milk fat concentration (Cannas et al., 1998; Zenou and Miron, 2008; Cannas et al., 2013; Lunesu et al., 2021). This is particularly important for dairy sheep, whose milk is mostly used to produce cheese.

For these reasons, the research described in this Chapter had the aim to:

a) define to which extent forage quality can affects milk production and composition during mid-lactation

b) quantify the effect of fiber quality on the forage intake of sheep during mid lactation.

3.2 MATERIAL AND METHODS

An experiment was carried out over 30 consecutive days, of which 7 were of pre-experimental period and 21 of experimental period, from the 21st May 2022 to the 21st June 2022, at the "Stalla didattico-sperimentale Mauro Deidda" (40° 46'N, 8° 29'E, altitude 89 m above sea level), University of Sassari, Department of Agricultural Science, Sassari, Italy. Experimental procedures used in the study were approved by the University Research Animal Ethics Committee.

3.2.1 Animals, feeding and experimental design

The experiment included 21 Sarda dairy ewes in mid lactation (5-6th months of lactation). After a pre-experimental period of 14 days, in which all the ewes were fed the high-quality alfalfa described in Table 1 and 600 g/d as fed of corn grains, three experimental groups were created. Groups were balanced for DMI, milk yield (MY), body weight (BW) and BCS, mean±SD; DMI 2.22±0.05 kg/d MY 2.00±0.03 kg/d; BW 53.9±2.04 kg; BCS 2.98±0.08Each group consisted of 7 ewes allocated to one of the three experimental dietary treatments for 21 consecutive days. The diet supplied during the pre-experimental period was made by dehydrated chopped alfalfa hay of high quality (AAH, with 23.5% CP, 39.0% NDF, 5.8% ADL, DM basis; Table 1) fed ad libitum, and by a supplementation of a fixed dose (528 g of DM/d) of whole corn grains. Daily concentrates were divided into two equal meals given at milking and forage refill was made at 08:00 am and 16:00 pm.

During the experiment, the animals were housed in a collective indoor pen (12 x 4 m) on wheat-straw bedding and had free access to fresh water, minerals and salt blocks. The straw intake was not recorded or taken into consideration in feed intake data, but the behavior was monitored daily to ensure that ewes didn't eat straw. The experimental diets were:

a) OH, made by dehydrated OH hay (7.3% CP, 63.9% NDF, 4.78% ADL, DM basis; Table 1) fed ad libitum and a supplementation of 176 g/d of whole corn grains and 360 g/d of soybean meal, DM basis;
b) AAM, made by dehydrated chopped alfalfa hay of medium quality (19.7% CP, 43.5% NDF, 6.48% ADL, DM basis; Table 1) and a supplementation of 528 g of DM/d of whole corn grains;
c) AAH, made by the same dehydrated chopped alfalfa hay of high quality (23.5% CP, 39.0% NDF, 5.8% ADL, DM basis; Table 1) used in the preliminary period, fed ad libitum and a supplementation of 528 g of DM/d of whole corn grains.

The soybean meal of the diet OH was used to compensate the low CP of the OH hay compared to the alfalfa hays of the other two treatments (Table 1).

3.2.2 Samplings and registrations

Dry matter intake (DMI) of forages was recorded daily and individually by using the Biocontrol AS (Norway) mangers, which allow the individual measurement of the intake of each animal, identified by a specific RFID. The concentrates were supplied individually at milking (6:30 am and 18:30 pm). Milk samples for milk quality analysis were taken weekly. The BW was recorded daily by an automatic precision scale. BCS was recorded weekly by three operators. Feed samples for chemical analysis were taken weekly. Concentrates were always completely eaten. Forage refusals were weighed daily for each group.

3.2.3 Chemical analysis and particle size measurements

The DM of feeds and refusals was determined weekly by drying the samples in a drying cabinet at 60 °C for 24 h. The dried feed samples were grinded in a millet with a grill diameter of 0.1 mm. The ground samples of feeds and refusals were analyzed for aNDFom (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP) and ash. The NDF was analyzed in an FiberTech fiber analyzer by use of heat stable α-amylase (Mertens, 2002; Van Soest et al., 1991). Results are expressed on an ash-free basis. The crude ash content was determined according to AOAC (2004). Total nitrogen content was analyzed using the Kjeldahl nitrogen determination according to AOAC (2004) and CP was calculated as total N x 6.25.

Calculations for the energy value of feeds

The Metabolizable energy (ME) and the Net energy of lactation (NEL) was calculated using the Small Ruminant Nutrition System equations (Tedeschi et al., 2010).

Particle size measurements

For the particle size measurements, it has been used the laboratory method with a vertical sieving "Endecotts Octagon 200 six-sieve model" with square mesh with diagonal sizes of 4.75 mm, 2.36 mm, 1.18 mm, 600 µm, 300 µm, 150 µm. The sieving method consisted of taking a sample of about 50 grams of forage. The sample was sieved for 30 min. After sieving was finished, the weight of each of the six particle size fractions was obtained for each sample directly by weighing in an analytical balance and in a precision balance for the size fractions between 2.36 and 1.18 mm and between 1.18 and 600 µm.

Measurement of potentially digestible NDF (NDFpd)

The NDFpd was estimated by subtracting from the NDF the proportion of ADL lignin, calculated as ADL x 2.4 (Van Soest, 1994) as follows:

$$\text{NDFpd}(\%\text{DM})=\text{NDF}(\%\text{DM})-(\text{ADL}(\%\text{DM})\times 2.4)$$

3.2.4 Statistical analysis

Data for DMI, NDF intake, BW, BCS, milk Yield and milk quality were analyzed statistically in a factorial design with feeding treatment, time and their interactions as fixed factors, and animals as random factor, using the Mixed model of R software (RStudio for Windows, version 4.4.0).

The following mathematical linear mixed model has been adopted:

$$Yij = \mu + D + T + D*T + ID + e$$

Where:

Yij = Observations of the variables corresponding to the repetition j under the treatment of order i;

μ = Overall average of observations;

D = Fixed effect of the Treatment;

T = Fixed effect of the period or sampling;

D*T = Interaction between fixed effects of treatment and period;

ID = random effect of the animal;

e = Random error.

For a significant F-test ($P<0.05$), pair-wise comparisons between least square means of treatments were performed according to Tukey's test. The P-values are shown in tables as: *** ($P<0.001$), ** ($P<0.01$), * ($P<0.05$), NS, no significance, $P>0.10$.

3.3 RESULTS AND DISCUSSIONS

In Table 2 is reported the chemical composition of the diet eaten by the ewes of the three experimental groups. On average, the AAM and AAH eat a diet with a higher concentration of CP (AAM=17.5% and AAH=21.0% vs OH=16.0% of DM) and a lower concentration of NDF (AAM=36.1% and AAH=33.9% vs OH=49.0% of DM) compared to the OH group. However, the AAM and AAH eat to a lower concentration of CP and NDF in respect to the one supplied with the forages, as reported in Table 1, while the OH group had eaten to higher values of CP and lower values of NDF in respect to those supplied with the forage as reported in Table 1. The differences among the groups in respect to the CP and NDF selection is in part due the differences in composition among the forages supplied, in part is due to the fact the diet was balanced to be isoproteic supplying fixe amount of concentrates and assuming a certain level of intake of the forages, but the actual intake of AAM and AMH was higher than the one expected. l. In fact, the OH hay had 7.32 % of CP and 63.9 % of NDF, while the AAM and AAH hay had 19.70% and 23.48 % of CP and the 43.5% and 39.0 % of NDF, respectively.
foraggi

3.3.1 Dry matter and forage intake

Table 3 reports the evolution of daily DMI of the diet and of the hay (Hay DMI) for the OH, AAM, and AAH groups over the course of the 21-day experimental period. These data are organized into time points, denoted as “T,” which aggregate measurements taken over three consecutive days. For instance, T1 represents measurements from days 1, 2, and 3, while T2 corresponds to days 4, 5, and 6, and so forth, with T7 encompassing measurements obtained from days 19, 20, and 21.

These data clearly show that over the duration of the trial, the three groups exhibited a statistically significant difference in their intake ($P<0.001$). Specifically, the AAH group had higher intake values for both total DMI and hay DMI when compared to the AAM group, which in turn exhibited higher intakes than the OH group. On average, the AAH group had a daily diet DMI of 2.73 kg/d, while the AAM group had 2.08 kg/d, and the OH group 1.29 kg/d only ($P<0.001$). This distinction in intake persisted throughout the trial, with the three groups having statistically differnt DMI values starting from T1: AAH 2.63 kg/d vs. AAM 2.18 kg/d vs. OH 1.15 kg/d ($P<0.001$) and continuing through T7: AAH 2.93 kg/d vs. AAM 2.10 kg/d vs. OH 1.29 kg/d ($P<0.001$). A statistically significant interaction between diet and days was observed, highlighting that the diet had a varying impact, more intense in the first experimental days, on intake across the 21-day period (Figure 1). The AAH group achieved its highest DMI during the T7 measurement, which marks the final phase of the trial, with intake levels reaching 2.93 kg/d (Table 3 and Figure 1). Conversely, the AAM group attained its peak DMI in the T4 measurement, with 2.19 kg/d and remained at similar levels in the rest of the trial (Table 3 and Figure 1). The OH group had a marked drop of DMI in the first experimental days, then it slowly increased until T5, reaching a maximum value of 1.49 kg/d, with a subsequent moderate reduction (Table 3 and Figure 1).

This pattern was almost identical focusing solely on hay intake (as presented in Table 3 and Figure 2), since all concentrates were supplied in a fixed dose and were completely eaten. The mean hay DMI of the AAH was 2.20 kg/d, for AAM was 1.51 kg/d, and for OH 0.75 kg/d ($P<0.001$; Table 3 and Figure 2). Maximum hay DMI coincided with the same measurement days as the total ration intake. As for diet DMI, statistically significant differences were observed for hay DMI from T1 (AAH 2.09 kg/d, AAM 1.65 kg/d, OH 0.62 kg/d, $P<0.001$) to T7 (AAH 2.39 kg/d, AAM 1.57 kg/d, OH 0.72 kg/d, $P<0.001$).

The fact that the AAH group had the highest total ration and hay intake can be attributed to the qualitative characteristics of the hay itself, which had lower concentration of NDF than the other hays (31.9% vs. 35.8% vs. 48.4% for AAH, AAM, and OH, respectively), thereby probably enabling animals to have high intakes due to a limited ruminal fill effect. This observation aligns with the findings of Osburn (1974), Van Soest (1994), Mertens (1994a), and Cannas (2004), suggesting that sheep modulate forage intake in response to its NDF content, a proxy of space occupied by the forage in the rumen. Furthermore, these results are consistent with the whole literature, which indicates that feeds characterized by high degradability and digestibility lead to increased intakes when compared with those featuring lower values (Macchioni et al., 1990). Additionally, the higher PG content found in high-quality alfalfa (23.48% vs. 19.70% vs. 7.32%, for AAH, AAM, and OH, respectively) may have stimulated its consumption due to its enhanced palatability.

3.3.2 NDF intake

The dietary effect was clear throughout the entire duration of the trial, demonstrating a high level of significance (Figure 3; $P<0.001$). Furthermore, a significant interaction between the diet and days was observed, indicating.

The total NDF intake, expressed in kg/d, is reported in Table 3 and Figure 3. At day T0, which marks the conclusion of the pre-experimental period, the ewes had a similar and nearly identical NDF intake (around 830 g/d per ewe), as they were all fed the same diet based on AAH ad libitum and 600 g/d of corn grains. However, as the experiment began and the diet was changed, immediate effects on DMI and NDFI become evident for the PAT and AAM groups, resulting in statistically significant differences in NDF intake (P<0.001).

Notably, there was a substantial decrease in the group OH groupOH, which halved its NDF intake, reaching values as low as of 0.466 kg/d with a subsequent recovery to 0.680 kg/d at T5, and then another reduction to an intake value of 0.532 kg, suggesting that the OH hay had a marked negative impact on DMI and NDF intake, probably for its high NDF concentration and ruminal filling mechanism (Allen, 2014; Mertens, 1994; Huhtanen et al., 2016).

In contrast, the group fed AAM experiences a less pronounced reduction in the initial phase, decreasing from 0.806 kg (T0) to 0.714 kg at the 3rd measurement, after which an oscillating pattern was observed. This group ended the trial (T7) with a NDF intake kg of 0.737 kg/d.

Conversely, the AAH group exhibited a consistently higher NDF intake throughout the experimental period compared to the other two groups. Its NDF intake followed a pattern of initial growth until the 2nd measurement, reaching a value of 0.963 kg/d, followed by a reduction and another increase at T5, reaching 0.928 kg/d. Subsequently, there was a decrease in intake, reaching a minimum of 0.825 kg, followed by a significant growth, with a value of 0.987 kg at T7. This can be attributed to the lower NDF of the AAH hay, and its lower ADL concentration when compared to the AAM hay. Likely, the AAH hay also had higher rumen degradability, which further reduced ruminal fill, allowing these animals to consume larger quantities. In contrast to the OH group, the AAH had the possibility to eat much higher quantities of NDF, since probably its NDF concentration was not a limiting factor for the intake, which was regulated by the ewes' requirements.

The trends observed for NDFI closely mirror the patterns of total dry matter intake and hay intake reported in Table 3, confirming that NDF intake increases with higher consumption of hay and dry matter.

3.3.3 Dry matter intake of NDF as percentage of body weight

As expected, the patterns of DMI and NDF intake as a percentage of body weight were similar between them (Table 3 and Figure 4). The already pronounced disparity between the groups observed for absolute DMI and NDFI further intensified, favoring the AAH group, which, on average, had a lower initial body weight than the other two groups.

The NDF intake as a percentage of body weight was statistically different among the groups (OH=1.14 vs AAM=1.33 vs AAH=1.68 NDFI%BW, P<0.01) throughout the study period. Additionally, the interaction between diet and measurement days was also significant.

The highest mean recorded value of NDF intake as a percentage of body weight was 1.81%, observed in the final measurement (T7) of the AAH. In contrast, the AAM group exhibited a maximum value of 1.41% at T1, followed by a subsequent decline. The OH group, on the other hand, reached its highest NDF intake as a percentage of body weight at T5, with a value of 1.32%.

These observed values for NDF intake as a percentage of body weight are below the maximum values reported by Cannas et al. (2016), Molle et al. (2014, 2016) and Nielsen (2016). This discrepancy may be attributed to differences in forage composition (e.g. Molle et al., 2014, 2016, had as forage source fresh grazed grass), and in stages of lactation and milk production level. Nonetheless, the data presented in this thesis are very useful also or a comprehensive assessment of the model proposed by Cannas et al. (2016).

AAHIn the OH group, animals displayed average NDF intake as a percentage of body weight ranging from 0.9 to 1.3%. These levels are comparable to those typically observed during the gestation phase, where intake as a percentage of body weight typically falls within the 1.0 - 1.1% range (Cannas et al., 2016), by Helander et al. (2014) and Olsen et al. (2016) for late gestation sheep, further highlighting the negative impact on intake during the lactation of OH hay.

3.3.4 Body weight and body condition score

Table 4 presents the body weight trends during the experimental period. It is noticeable that all three groups experienced a weight loss during the initial week of the trial (Figure 5), possibly attributable to the dietary change-induced stress, since in the first days the ewes had to learn where to eat. Subsequently, weight gains were observed in all groups in the ensuing measurements. The AAM group exhibited the most pronounced weight increase, with a gain of +2.3 kg during the 21 d, progressing from 55.1 kg at T1 to 57.4 kg at T7. The AAH group, starting at 53.7 kg at T1, recorded a weight of 55.9 kg during the final 3 days, resulting in a gain of +2.2 kg over the 3-week period. Conversely, the OH Hay (OH) group showed the smallest weight gain, +1.5 kg, transitioning from 50.9 kg at T1 to 52.4 kg at T7.

Despite the numerical differences in weight gains, the diets did not exert a statistically significant effect on them. Similarly, there was no significant interaction between the diet and time. The observed weight increase during this study is probably not directly tied to the quality of the ingested hay and might be due to changes in rumen fill. In alternative, a physiological explanation for these findings could be that the animals reduced their requirements for milk production and increased their reserves. Consequently, the two groups with access to higher-quality hay, AAH and AAM, could eat more energy than that required for maintenance and milk production. In contrast, the OH group was limited by the poorer quality of the hay and was unable to accumulate body reserves.

Table 4 and Figure 6 reports the evolution of BCS during the experimental period, with T0 marking the final day of the pre-experimental phase and T7 the last 3 experimental days. The data reveal that throughout the experimental period the BCS did not follow the same trajectory of body weight. Indeed, the BCS tended to decrease, declining from 2.95 (T0) to 2.88 (T7) for the AAH group, from 3.05 (T0) to 2.89 (T7) for the AAM group, and from 2.95 (T0) to 2.77 (T7) for the OH group. This reduction, although not statistically significant, was most noticeable in animals fed the lowest-quality hay diet. The AAH group had a much more limited BCS loss compared to the OH group, likely because of its higher hay intake, which provided more energy and reduced the need to mobilize body reserves. The weight gain previously observed in the OH group suggests that it may have stemmed from increased rumen fill and water intake in the last two weeks of the study, rather than on body fat deposition. Consistent with weight data, the only significant factor influencing BCS was time ($P<0.05$).

3.3.5 Milk Yield

Table 5 displays the evolution of daily milk production of the three experimental groups over the course of the study period, while Table 6 presents the corresponding values on the dates in which there was also the measurement of milk composition. The comparative analysis of the data highlighted, throughout the whole experiment, significant differences in milk production among the three groups.

Specifically, the AAH group consistently had higher milk production levels ($P<0.001$), with an average of 2.03 kg/d, in contrast to the AAM group, which averaged 1.76 kg/d. The AAM group, in turn, consistently outperformed the OH group, which had an average production of 1.49 kg/d. An almost constant production trajectory was observed in the AAH group, with a reduction in production occurring only in T7. In contrast, the AAM group displayed a continuous decline in production, going from 1.95 kg/d at T0 to 1.72 kg/d by T7. The OH group had an even more marked decline in production, going from 2.01 kg/d at T0 to 1.36 kg/d T7.

These differences and patterns can be attributed mostly to dietary effects of dietary factors, since while the AAH groups had a constant milk production, suggesting that the ewes were not yet in the declining stage of milk production, the other two reduced it over time, which a significant temporal effect ($P<0.01$). In particular, the OH group, which had the lowest quality diet, exhibited a marked decline in production compared to the other groups. This decline reflects the decline in DMI and NDI intake observed during the experiment for the same group, even though milk production kept declining even when DMI stabilized and partially recovered from T3 to T7 after its initial marked reduction (Table 2 and Figure 2).

When comparing these results with those of Peana et al. (2007) who used two different diets for NDF% (high 49.4 and low 35.4 %NDF) and similar groups for MY (1.95 kg/d) we observed that in our study the level of NDF of the diet had a significant effect both on MY and DMI while none effect of the diet was observed by Peana et al. (2007). It is possible that the effects of the diets might be covered in the case of Peana et al. (2007) by the heatstress which in fact create a decrease of DMI and MY in their study.

Also, in a study conducted by Cannat et al. (2013) in which two levels of NFC were tested (high 36% and low 23%), the highest MY was reached with the diet that had less NFC (NFC23) and a level of 50.6% of NDF. However, in the study of Cannas et al. (2013) the differences between the groups are not reliable to the sole NDF but also to the fact that the NFC36 group had an higher percentage of starch in the diet compare to NFC23, that has been used probably as fat deposit instead of being used as dietary energy towards milk synthesis. To underline that, the utilization of high digestible fiber in mid lactation had a positive impact on MY. In fact, also in our study the AAH group which ate the highest quality of forage and the lowest amount of starch (13.1% of starch) had the highest MY (2.02 kg/d). This findings are also in line with the studied reported by Bovera et al. (2004) and Zenou and Miron (2005) which obtained the highest level of MY (2.40 kg/d) when substituting the NFC of the diet with increasing levels of NDF made mostly from byproducts (i.e. beet pulp and soybean hulls).

3.3.6 Milk composition

Table 6 reports the chemical composition of milk obtained from the three different diets.

The effect of diet on milk fat percentage was significant (P<0.01), indicating that it was influenced by the quality of the diet and the forage, while Time had not a significant effect. The average fat content was significantly higher in the OH group compared to the AAM group, which in turn had a significantly higher content than the AAH group: OH 7.24%; AAM 6.93%; AAH 6.44% (P<0.01). The fat percentage in the milk was higher in the OH group, compared to the other groups, from T1 throughout the end of the experimental phase (Figure 8). This parameter showed an increasing trend in the OH group, in contrast to the AAM group, which had a almost constant milk fat concentration during the whole experiment. On the other hand, the AAH group progressively reduced its milk fat concentration, with a partial recovery only in the last sampling period (T7). In the AAH group, the lower fat percentage is likely due to dilution effect, because these animals had higher milk production compared to the other two groups. Conversely, the higher values in the OH group are attributed to the opposite effect, i.e. of concentration of milk fat due to the reduction in milk yield.

Milk fat yield was significantly affected by the diet (P<0.05), with no significant effects of time or the interaction between time and diet (Table 6). Fat yield was consistently higher throughout the trial in the AAH group (P<0.05), due to its higher milk yield and despite its initially (T0) lower fat yield, compared to the AOT group, while the AAM was intermediate and did not differ from the other two groups. The mean values of milk fat yield of these groups reflected the trends observed during the trial, with the AAH group having an average of 125 g/d, the AAM group of 116 g/d, and the OH group of 105 g/d. The difference in fat content in grams between the AAH and AAM groups compared to OH was statistically significant.

The milk protein percentage, like fat, was numerically higher in the OH group and lower in the AAH group, characterized by higher production (Table 6 and Figure 9). These difference, however, were not significant, like those of time, or the interaction between diet and time.

Regarding the daily production of milk protein, there was a highly significant effect with diet (P<0.001), while time and the interaction between diet and time were not significant. Protein yield was higher in the group of animals that produced more milk, i.e., that fed AAH, which had an average protein yield of 110.8 g/day, significantly higher than the AAM group's mean of 96.2 g/day and even higher than that of the OH group (OH) at 83.2 g/day. This could be due to a more energy-rich diet, which allowed for greater milk protein synthesis, in the AAH compared to the highly fibrous OH diet.

Regarding urea in milk, there was a highly significant effect of diet (P<0.001), a significant effect of time (P<0.01), and significant interaction between diet and time (P<0.05). The highest mean values (at 41.9 mg/dl) were observed in the AAH diet group, probably due to its higher DMI and, thus, intake of protein. . The milk urea concentration observed aligns with what is reported in the literature by Cannas et al. (1998), highlighting that urea content in milk or blood varies with changes in protein concentration in the diet and intake in the diet.

The lactose content was affected by the diet (P<0.05) but not by the time effect, and the interaction between diet and time. The OH group decreased its lactose content compared to T0, while the other two groups had a stable value.

Overall, it appears that although the AAH group had a lower concentration of fat, protein, and casein, it produced greater quantities of milk components suitable for cheesemaking compared to the other two groups.

For all the three groups, OH, AAM and AAH we observed highest values for fat and protein percentage and fat yield compared to those reported by Cannas et al. (2013), while we observed similar values for the protein yield. Such differences might be explained by the fact that the source of NDF between the two studies compared where different. In fact, in our study the main source of NDF was represented by the forages while for Cannas et al. (2013) was represented from the alfalfa pellet and the byproduct used (beet pulp and soybean hulls). Thus, the forage in our case may have caused a highest amount of precursor of fatty acid such as Acetic Acid. On the other hand, the levels of Urea of our study are lower compared to those of Cannas et al. (2013) even if, interestingly, the concentration of CP of the AAH diet were also higher compared to those used in the study of Cannas et al. (2013). A partial explanation to this difference might be that more protein in our study has been used to create milk protein and thus that at least the group AAH had a better ruminal microbial synthesis explained by the low level of Urea in the milk but at the same time to the high productive level and high synthesis of milk fat and milk protein. Closer values to ours of milk fat percentage and milk fat yield were reported by Zenou and Miron (2005) but in mid lactating ewes of different breed (Assaf).

3.4 CONCLUSIONS

The quality of forage significantly influenced the DMI of the animals. The AAH group had the highest DMI, followed by AAM, and OH. The higher intake in the AAH group can be attributed to its better quality (lower NDF and higher PG), which enhances palatability and digestibility. The effect of the diet was consistent throughout the trial and had a statistically significant impact on all three experimental groups. The quality of the diet also significantly affected the NDF intake, with AAH having the highest intake, followed by AAM, and OH. The NDF intake patterns closely mirrored total dry matter and hay intake, confirming that NDF intake increases with higher consumption of dry matter and forage when a forage-based diet is supplied. The AAH group also had the highest NDF intake as a percentage of body weight, with the lowest body weight, highlighting the impact of diet quality on this parameter. The OH group had consistently lower levels of NDF intake, which for this diet was closer to the typical range for the gestation phase and thus much lower than the typical values of the lactation stage, underlining the negative effect that mature forage have at rumen level in terms of fill effect.

All three groups experienced initial weight loss, possibly due to dietary change-induced stress, followed by weight gains over the 21-day trial. The BCS decreased during the experimental phase, with the OH group showing the most substantial decline, indicating a potential need to mobilize body reserves due to poor-quality hay.

The AAH group had the highest and more stable milk production, followed by AAM and OH groups. Time had a significant impact on milk production, with production decreasing over the course of the 21-day trial in both AAM and OH. The OH group experienced a more pronounced decline in milk production, likely due to the lower quality of the hay and the subsequent reduced intake.

The quality of the diet significantly affected the chemical composition of milk, with the OH group having higher fat and protein percentages, but the AAH group had highest milk fat and protein yield, and thus highest quantities of milk components suitable for cheesemaking. Urea content in the milk was highest in the AAH group, probably because its highest intake of protein. Lactose content in milk was significantly influenced by the diet even if pre-existing differences were evident since T0.

In summary, the study demonstrates a marked impact of forage quality on animal intake, milk production, and milk composition. The high-quality alfalfa hay resulted in the highest DMI, milk production, milk protein and milk fat yield, while the OH hay had the lowest values.

These findings provide valuable insights into the relationships between diet, intake, and milk quality during mid lactation, highlighting the importance of forage quality for optimizing livestock nutrition and production.

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3.6 TABLES AND FIGURES

Table 1. Chemical composition of hays and concentrates used in the formulation of lactation rations. OH = OHs, AAM = medium-quality alfalfa and AAH = high-quality alfalfa.

	OH hay	**AAM hay**	**AAH hay**	**Corn grains**	**Soybean meal**
DM, % as fed	87.23	88.31	89.38	88.00	90.00
CP, % DM	7.32	19.70	23.48	9.80	49.90
NDF, % DM	63.87	43.53	39.02	10.00	14.90
ADF, % DM	35.85	30.69	27.19	-	-
ADL, % DM	4.78	6.48	5.77	0.22	0.31
Starch, % DM	-	-	-	73.88	2.14
NFC, % DM	34.30	41.5	35.80	75.80	28.90
Ash, % DM	9.03	8.71	9.84	1.60	7.20
EE, % DM	2.30	2.40	2.60	4.30	1.60
Mineral composition*					

Mineral composition*: Ca = 146 g, P = 30 g, Mg = 38 g, Na = 130 g, I = 50 mg, Mn = 1.800 mg, Se = 8,3 mg, Zn = 2.880 mg, Vitamin A = 500.000 U.I., Vitamin D3 = 50.000 U.I., Vitamin E = 1244 U.I., Vitamin B1 = 15 mg, Vitamin B2 = 18 mg, Vitamin B6 = 9 mg, Vitamin B12 = 52,7 mg

Tabella 2. Chemical composition of the diet selected by the ewes during the 21 days of experiment. OH = OHs, AAM = medium-quality alfalfa and AAH = high-quality alfalfa.

Variable	**DIET**		
	OH	**AAM**	**AAH**
Chemical composition and energy value			
DM, % as fed	87.23	88.31	89.38
CP, %DM	16.0	17.5	21.0
NDF, %DM	49.0	36.1	33.9
ADL, %DM	4.78	6.48	5.77
Starch, %DM	17.8	16.4	13.1
NFC, %DM	26.9	39.5	37.0
Ash, %DM	8.0	7.1	8.4
EE, %DM	2.40	2.8	2.9
ME, Mcal/kgDM[1]	2.284	2.411	2.406
NEL, Mcal/kgDM[1]	1.471	1.553	1.550
Particle size retained in each sieve, % of the total			
Mesh diameter in mm			
4.750	72.00	52.00	22.00
2.360	8.00	12.00	26.00
1.180	12.00	12.00	26.00
0.600	6.00	8.00	12.00
0.300	2.00	8.00	8.00
0.150	0.00	6.00	4.00
<0.150	0.00	2.00	2.00
Total	100.00	100.00	100.00

[1] ME and NE_L, energy was calculated using the Small Ruminant Nutrition System equations (Tedeschi et al., 2010).

Table 3. Evolution of total dry matter intake (DMI), and hay intake (Hay_DMI), expressed as kg/d, for the three types of diets: OHs (OH), medium quality alfalfa (AAM), and high quality alfalfa (AAH) during the 21 test days. T= survey days grouping, 3 survey days each.. T0 = values at the end of the pre-experimental phase.

	Diet	**Time sampling**								**Mean**	**SE^{a}**	**P-value**		
		T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**			**D^{b}**	**T^{b}**	**D*T^{b}**
DMI, kg/d	OH	2.403	1.153	1.115	1.230	1.391	1.487	1.392	1.256	1.289^{C}	0.119	***	0.06	***
	AAM	2.435	2.183	2.051	1.951	2.189	2.010	2.104	2.104	2.085^{B}	0.119			
	AAH	2.630	2.629	2.865	2.735	2.686	2.775	2.512	2.927	2.733^{A}	0.119			
Hay_DMI, kg/d	OH	1.803	0.619	0.581	0.696	0.857	0.953	0.858	0.722	0.755^{C}	0.119	***	0.06	***
	AAM	1.835	1.649	1.517	1.417	1.655	1.476	1.570	1.570	1.551^{B}	0.119			
	AAH	2.030	2.095	2.331	2.201	2.152	2.241	1.978	2.393	2.199^{A}	0.119			
NDFI, kg/d	OH	0.820	0.466	0.442	0.515	0.618	0.680	0.619	0.532	0.587^{C}	0.016	***	*	***
	AAM	0.806	0.771	0.714	0.670	0.774	0.696	0.737	0.737	0.738^{B}	0.006			
	AAH	0.871	0.871	0.963	0.912	0.893	0.928	0.825	0.987	0.903^{A}	0.007			
NDFI, %BW	OH	1.58	0.92	0.88	1.02	1.21	1.32	1.19	1.02	1.14^{C}	0.029	**	NS	***
	AAM	1.44	1.41	1.31	1.22	1.39	1.24	1.30	1.30	1.33^{B}	0.010			
	AAH	1.58	1.64	1.80	1.73	1.67	1.71	1.51	1.81	1.68^{A}	0.013			

[a] SE=standard error of the mean

[b]T=sampling day, D=diet, D*T=interaction diet*sampling day
*P<0.05, **P<0.01, ***P<0.001, NS=not significant

Table 4. Evolution of body weight, expressed in kg, and BCS for the three types of diets: OH (OH), medium quality alfalfa (AAM), and high-quality alfalfa (AAH) during the 21 test days. T= survey days grouping, 3 survey days each. T0 = weight and BCS at the end of the pre-experimental phase.

		Time sampling								**Mean**	**SE[a]**	**P-value**		
	Diet	**T0**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**			**D[b]**	**T[b]**	**D*T[b]**
BW, kg	OH	51.8	50.9	50.9	50.8	51.6	51.9	52.5	52.4	51.6	1.94	NS	***	NS
	AAM	55.9	55.1	55.5	55.3	56.1	56.5	57.1	57.4	56.1	1.94			
	AAH	54.0	53.7	53.8	53.8	54.5	55.2	55.8	55.9	54.7	1.94			
BCS (0-5)	OH	2.95	-	2.91	-	-	2.84	-	2.77	2.87	0.13	NS	*	NS
	AAM	3.05	-	2.89	-	-	2.95	-	2.89	2.94	0.13			
	AAH	2.95	-	2.89	-	-	2.93	-	2.88	2.92	0.13			

[a] SE=standard error of the mean

[b] T=sampling day, D=diet, D*T= interaction diet*sampling day

*P<0.05, **P<0.01, ***P<0.001, NS=not significant

Table 5. Evolution of daily milk yield (MY), in kg/d, for the three types of diets: OH (OH), medium quality alfalfa (AAM) and high-quality alfalfa (AAH) during the 21 test days. T= survey days grouping 3 survey days each. T0 = MY at the end of the pre-experimental phase. T= survey days grouping, 3 survey days each.

	Diet	**Time sampling**									**P-value**			
		T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean**	**SE**^{a}	**D**^{b}	**T**^{b}	**D*T**^{b}
MY, kg/d	OH	2.009	1.705	1.740	1.574	1.401	1.277	1.363	1.379	1.491^{C}	0.062	***	**	NS
	AAM	1.948	1.912	1.811	1.790	1.703	1.674	1.678	1.716	1.755^{B}				
	AAH	2.006	2.081	2.069	2.140	2.044	1.965	2.023	1.816	2.028^{A}				

[a] SE=standard error of the mean

[b] T=sampling day, D=diet, D*T= interaction diet*sampling day

*P<0.05, **P<0.01, ***P<0.001, NS=not significant

Letters in the mean of the data indicate significant differences for P<0.01

Table 6. Chemical composition of milk according to the qualitative analysis by the LAORE laboratory in Oristano, Italy, for the three types of diets: OH (OH), low quality medica (AAM), and high quality medica (AAH) during the 21-day trial. T0 = values at the end of the pre-experimental phase. T= survey days grouping, 3 survey days each

	Diet	**Time sampling #**				**Mean**	**SE^{a}**	**P-value**		
		T0	**T2**	**T5**	**T7**			**D^{b}**	**T^{b}**	**D*T^{b}**
MY, kg/d	OH	1.847	1.517	1.496	1.307	1.440^{b}	0.063	***	NS	NS
	AAM	1.693	1.698	1.711	1.667	1.692^{ab}				
	AAH	1.841	2.029	2.066	2.028	2.041^{a}				
Fat, %	OH	7.05	7.05	7.29	7.59	7.24^{a}	0.119	**	NS	NS
	AAM	7.01	6.73	7.08	6.90	6.93^{ab}				
	AAH	6.63	6.45	6.15	6.52	6.44^{c}				
Fat yield, g	OH	130.1	106.6	108.8	99.7	105^{b}	2.01	*	NS	NS
	AAM	116.8	113.5	120.2	113.5	116^{ab}				
	AAH	119.3	125.1	122.4	127.0	125^{a}				
Protein, %	OH	5.92	5.76	5.83	5.81	5.80	0.020	NS	NS	NS
	AAM	5.75	5.65	5.82	5.71	5.73				
	AAH	5.64	5.42	5.52	5.61	5.52				
Protein yield, g	OH	109.6	86.8	86.8	76.0	83.2^{b}	3.00	**	NS	NS
	AAM	96.5	95.4	99.1	94.1	96.2^{ab}				
	AAH	102.3	108.7	112.1	111.6	110.8^{a}				
Fat/Protein	OH	1.20	1.23	1.26	1.31	1.27^{a}	0.015	**	NS	NS
	AAM	1.22	1.19	1.21	1.21	1.20^{ab}				
	AAH	1.17	1.18	1.11	1.16	1.15^{b}				
Lactose	OH	4.91	4.89	4.86	4.72	4.82^{b}	0.02	***	NS	NS
	AAM	5.01	4.99	5.00	5.03	5.01^{a}				
	AAH	4.88	4.84	4.88	4.88	4.87^{b}				
logSCC	OH	2.345	2.33	2.05	2.23	2.28	0.068	NS	NS	NS
	AAM	2.103	2.27	2.00	2.10	1.99				
	AAH	2.027	2.23	1.93	2.28	2.20				
Casein, %	OH	4.58	4.45	4.52	4.51	4.49	0.06	NS	NS	NS
	AAM	4.47	4.37	4.53	4.43	4.44				
	AAH	4.32	4.14	4.22	4.30	4.22				
Casein yield, g	OH	84.8	67.0	67.2	59.1	64.4^{b}	2.27	***	NS	NS
	AAM	74.9	73.8	77.1	73.0	74.6^{ab}				
	AAH	78.2	82.8	85.5	85.5	84.6^{a}				
Urea, mg/dL	OH	42.6	37.5	34.5	25.2	32.4^{b}	0.91	***	*	NS
	AAM	38.4	39.0	35.3	33.3	35.9^{b}				
	AAH	44.0	40.2	41.7	41.9	41.3^{a}				

ǂ values of the day within each period of 3 days in which milk was sampled for analysis;
SE= standard error of the mean [b]T = Time sampling, D=diet, D*T= interaction diet*sampling day
* P<0.05, **P<0.01, ***P<0.001, NS=not significant.
Letters in the same column for the mean of the same variable indicate significant differences for P<0.05

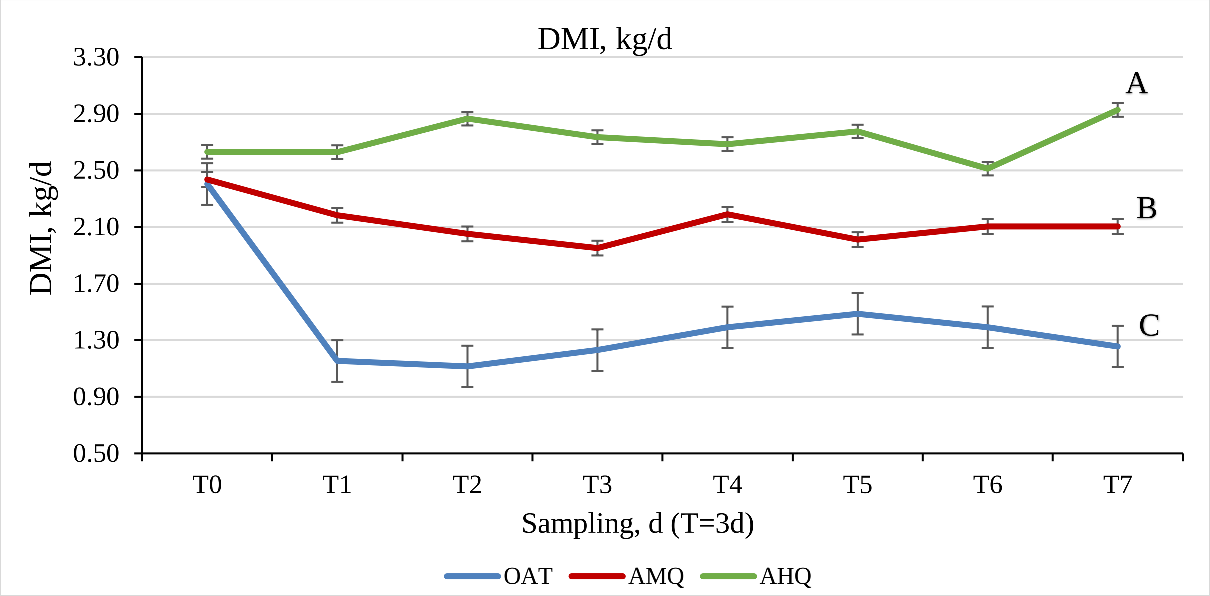

Figure 1. Evolution of DMI for the 3 experimental groups OH, AAM, and AAH, during the 21 test days, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each. Letters indicate significant differences among the means for P<0.01.

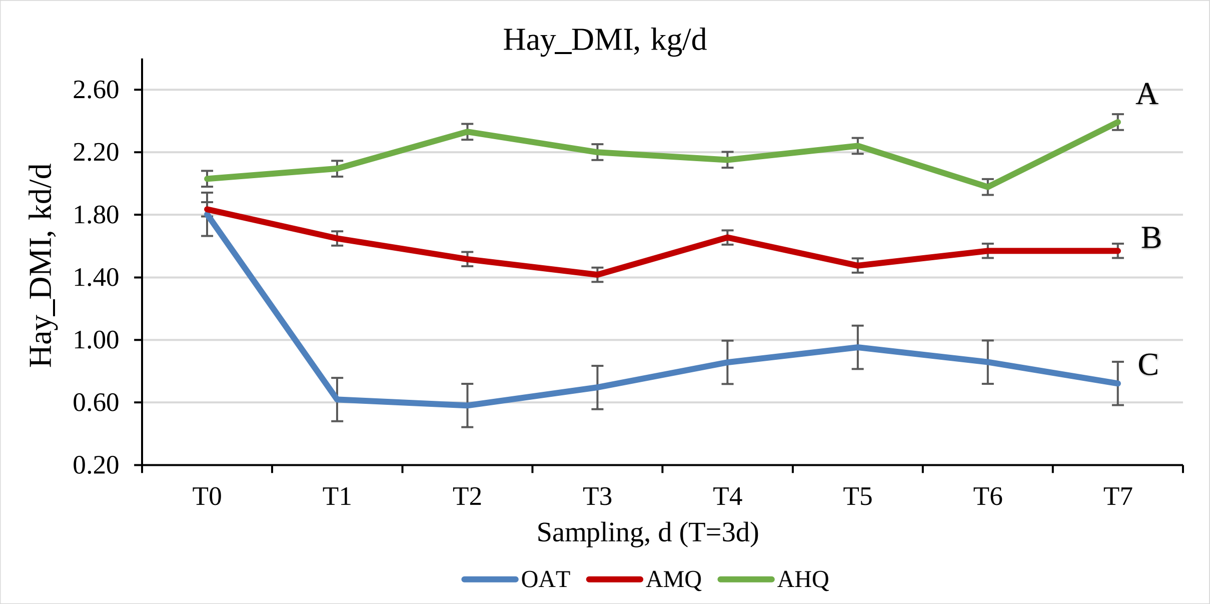

Figure 2. Evolution of DMI of hay for the 3 experimental groups OH, AAM, and AAH, during the 21 test days, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each. Letters indicate significant differences among the means for P<0.01.

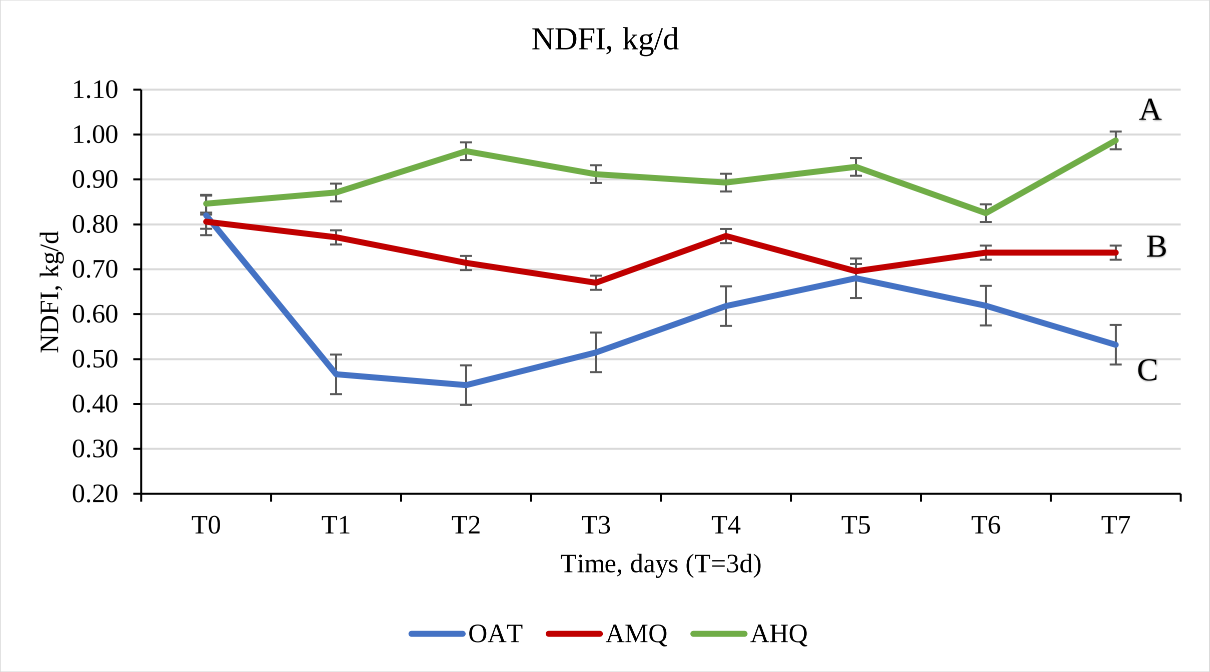

Figure 3. Evolution of NDF intake (kg/d) for the 3 experimental groups OH, AAM, and AAH, during the 21 test days, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each. Letters indicate significant differences among the means for P<0.01.

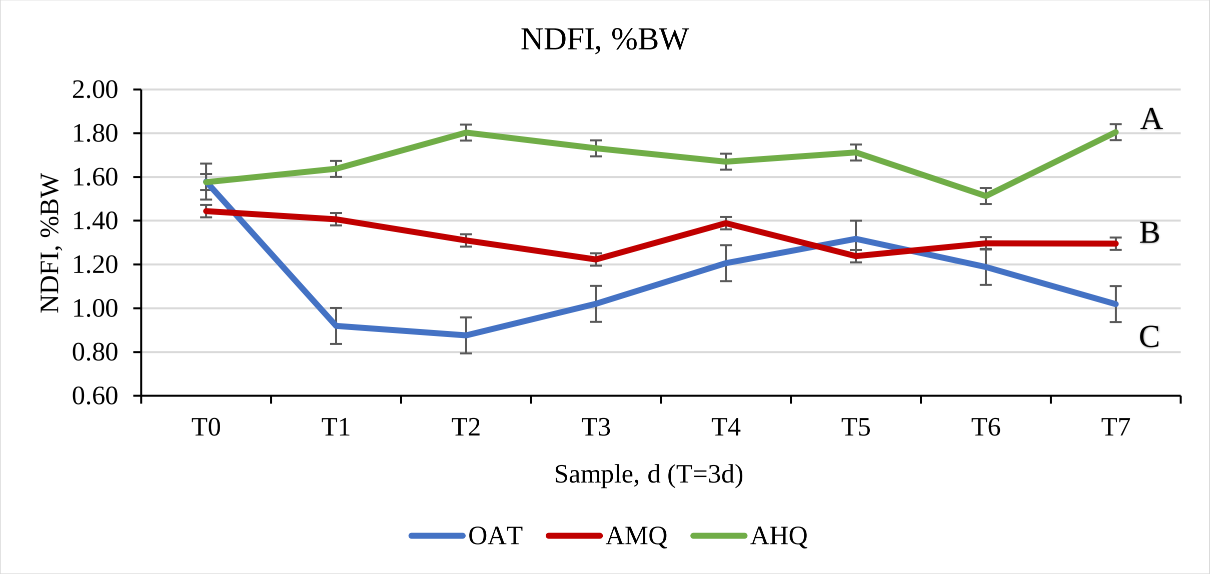

Figure 4. Evolution of intake of NDF express as percentage of BW for the 3 experimental groups OH, AAM, and AAH, during the 21 test days, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each. Letters indicate significant differences among the means for P<0.01.

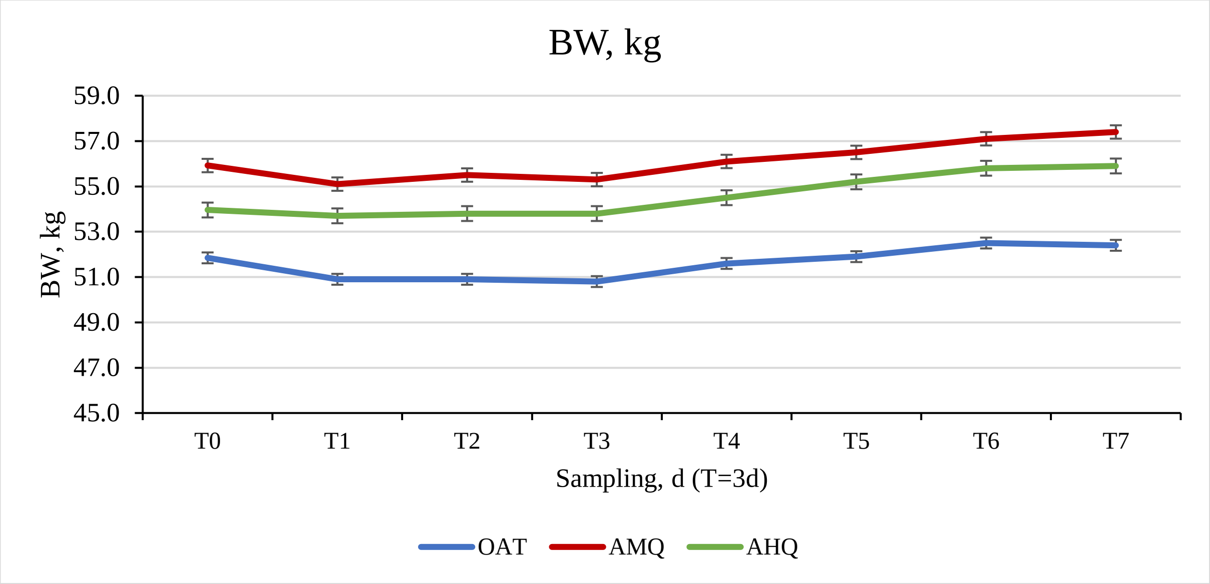

Figure 5. Evolution of body weight for the 3 experimental groups OH, AAM and AAH, during the 21-day trial, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each.

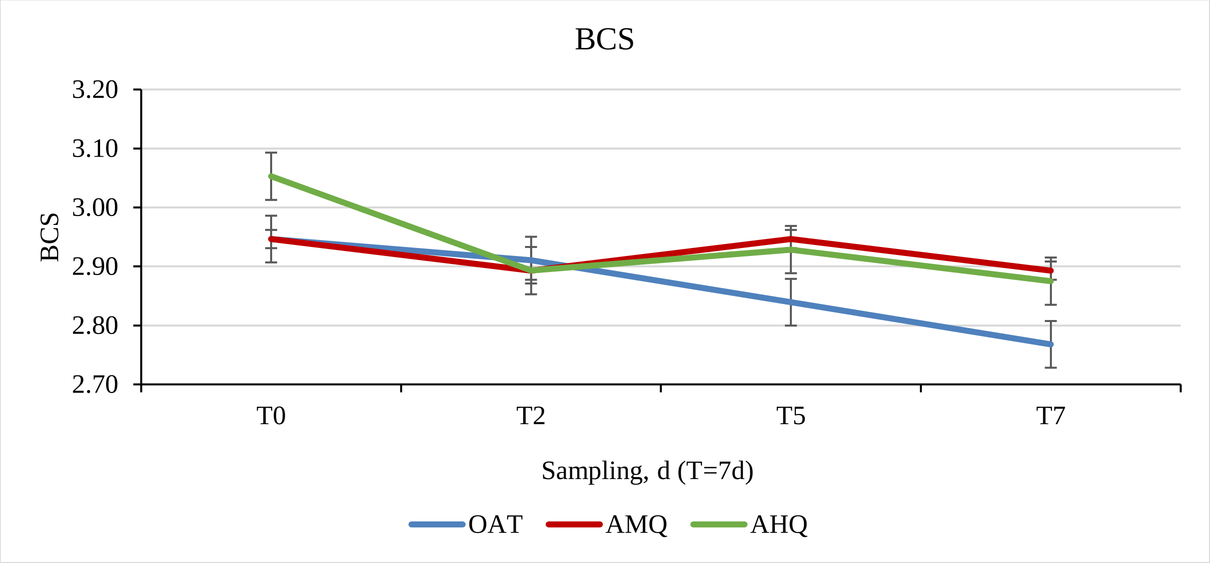

Figure 6. Evolution of BCS for the 3 experimental groups OH, AAM and AAH, during the 21-day trial where T0 is the BCS at the last week before the differentiation of diets and where each T = 7d of measurements.

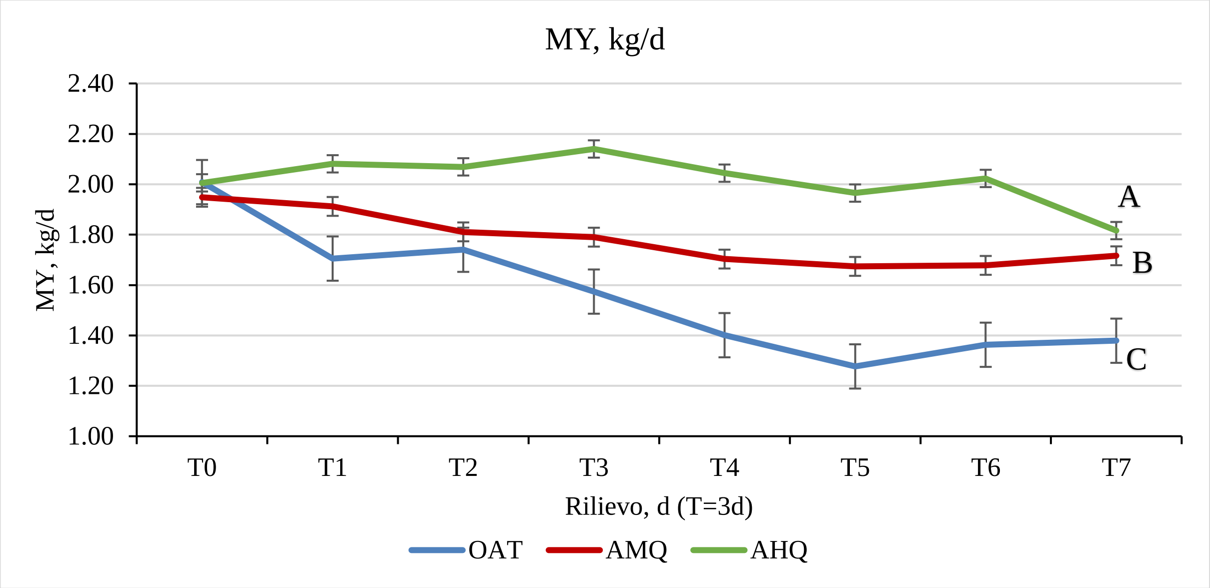

Figure 7. Evolution of milk yield for the 3 experimental groups OH, AAM and AAH, during the 21 test days, where T0 is the average of the three last preliminary day, and T = mean of 3 measurement days each. Letters indicate significant differences among the means for $P<0.01$.

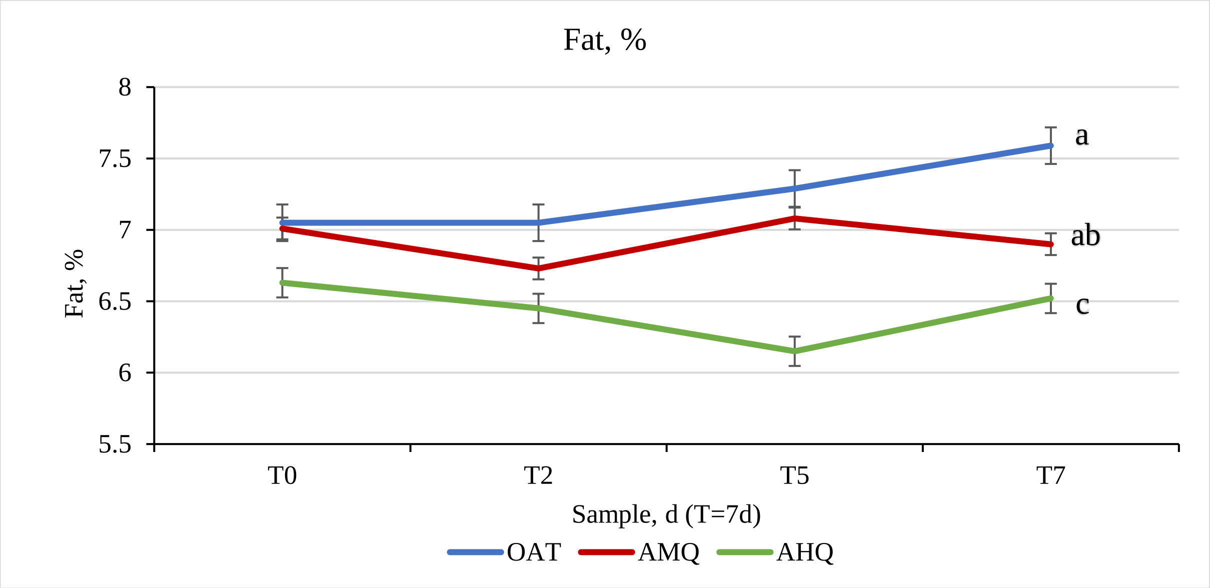

Figure 8. Evolution of milk fat percentages for the 3 experimental groups OH, AAM and AAH, during the 21-day trial, where T0 is the fat percentage at the last week before the differentiation of diets and where each T = 7d of measurements. Small letters indicate significant differences among means for $P<0.05$.

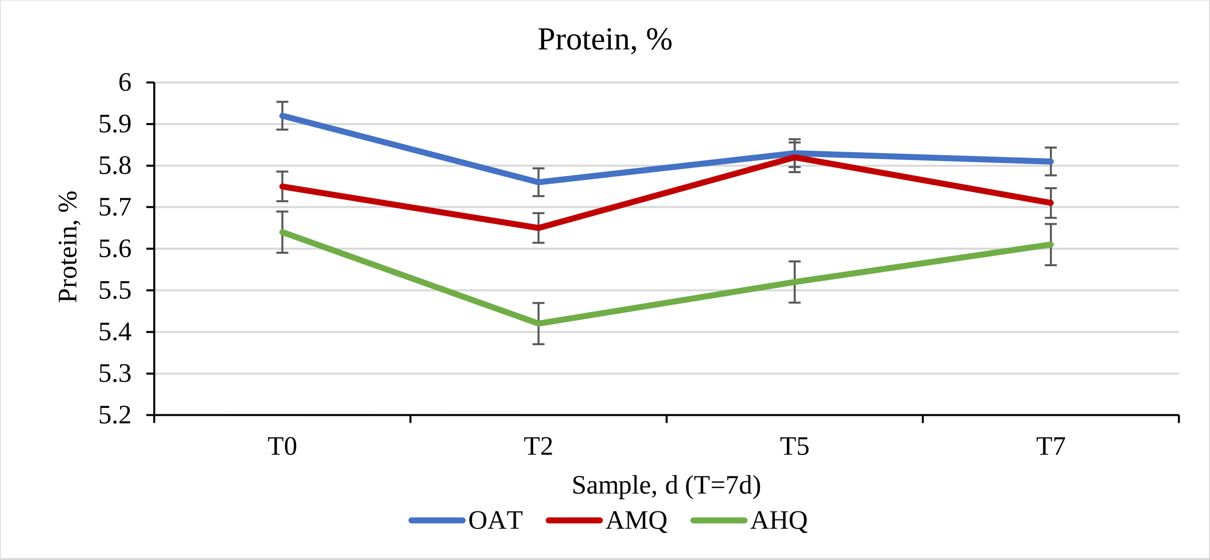

Figure 9. Evolution of milk protein percentages for the 3 experimental groups OH, AAM and AAH, during the 21-day test period, where T0 is the protein percentage at the last week before the differentiation of diets and where each T = 7d of measurements.

4 CHAPTER 4

Evaluation of prediction models of voluntary dry matter intake of Sarda ewes in the transition stage of late pregnancy and early lactation

ABSRACT

Existing DMI prediction models were evaluated in order to validate observed DMI in Sarda dairy ewes fed diets with different forage sources during late pregnancy and early lactation. Twenty-eight pregnant Sarda dairy ewes were assigned based on body weight, days in pregnancy and litter number (BW, mean±SD; 54±8.7 kg; 115±2 DIP; 1.24 litter number), to one of the two experimental diets and fed until parturition. One diet (OH) was composed by ad libitum dehydrated chopped oat hay (NDF 65.6%) and a fixed amount of concentrate (350 g/d of whole corn grains and 140 g/d of soybean meal, DM basis), while the other diet (AAM) was composed by ad libitum dehydrated chopped alfalfa hay (NDF 53.3%) and a fixed amount of concentrate (440 g/d of whole corn grains, DM basis). Following the pregnancy phase, the same ewes (n=25, BW, mean±SD; 51.02 ± 2.1 kg; MY; mean±SD; 2.45±0.45 kg/d) were fed the same AAM diet (NDF 53,3% and CP 14.7%, DM basis) and a fixed amount of concentrate (440 g/d of whole corn grain + 135 g/d of soybean meal, DM basis) within the first 28 days of lactation. Three DMI models (Pulina et al., 1998, Model 1; Gallo and Tedeschi, 2021, Model 2; and Serra, (1998), Model 3) for gestating and lactating ewes were compared to observed DMI and evaluated using the software Model Evaluation System version 2.3.4. We correct the model of Serra, (1998) with the continuous adjusted factor (fDMI) proposed by Gallo and Tedeschi, 2021 as a Model 4 to be evaluated. For dairy ewes during late pregnancy, Model 1 overestimated the DMI of the OH group (P-O= 0.1 kg/d), while underestimated that of the AAM group (P-O= -0.20 kg/d; P<0.01). Model 2 underestimated DMI for the AAM group (P-O= -0.24; P<0.01), while the mean bias was very low for the OH group (P-O= -0.03 kg/d; P>0.1). DMI in early lactation was markedly overestimated by the 3 models, both in AAM and OH group. Based on our results, Model 4 (Serra, 1998 adjusted) was the most precise and accurate in fitting the observed DMI in early lactation in both groups, AAM (r2=0.82, CCC=79, Cb=0.86 and RMSEP=0.20, P-value<0.001), and OH (r2=0.91, CCC=79, Cb=0.83 and RMSEP=0.20, P-value<0.001). These findings suggested that more observed data are necessary for pregnancy and lactating ewes to be tested in DMI prediction models. More mechanistic models, including predictors associated to dietary factors CP and NDF of the diet and rumen size and fill, should be developed.

Keywords: DMI, nutrition model, pregnancy, lactation, mathematical model, ewes

4.1 INTRODUCTION

Dry matter intake (DMI), considered an input when measured, or an output, if estimated by models (Busanello et al., 2021), is widely used as an indicator of farm income (Roseler et al., 1997), animal health and estrus status (Lukas et al., 2009), and performance in dairy cattle (Lee et al., 2021). International bodies (CSIRO, 1990; INRA, 2007, NRC, 2007) and other researchers (Cannas et al., 2004; 2010; Tedeschi et al., 2010; Tedeschi and Fox, 2018) have provided detailed feed intake prediction tables and estimation equations upon which current sheep feeding systems have been adjusted and can be referenced (Avondo, 2005).

Diet intake control is multifactorial, involving a wide range of animal factors, such as sex, body weight, age, breed (NRC, 2007), physiological stage (growth, gestation, or lactation) and metabolic factors (Mertens, 1994; Allen 2000; Oliveira et al., 2014), feeding behavior (Halachmi et al., 2016; Liang et al., 2021), environments factors, such as heat stress (Hill and Wall, 2017), feed and diet factors, such as CP and NDF content and degradability, fat content, presence of indigestible fractions or of secondary compounds, particle size, palatability (Van Soest, 1994; de Oliviera et al., 2020), and, in grazing animals, pasture characteristics (Carvalho, 2013).

Regarding the estimation of DMI during late gestation or early lactation, the factors affecting feed intake are variable over time. Pulina et al. (1993) showed how high dry matter intake can persist even when milk production declines as lactation progresses. This is likely related to the physiological need of sheep to replenish body reserves depleted during the initial weeks following parturition (Avondo, 2005). During gestation, a shift from metabolic intake control to physical intake regulation, due to rumen fill, is generally observed as pregnancy progresses (Trabalza-Marinucci et al., 1992). As an example, in late gestation, decrease in voluntary hay DMI was observed in Sarda ewes fed high forage to low concentrate diets (Trabalza-Marinucci et al., 1992). Although DMI decreases with advancing pregnancy (Rotta et al., 2015), Bocquier et al. (1987) suggested that in late pregnancy there is more a change in the mechanism of regulation of the substitution rate between forages and concentrates than a real decrease in intake capacity of ewes.

The measurements of DMI on commercial ruminant farms relies heavily on manual labor, making daily recording laborious and prone to errors even when done at group level. As a result, intake measurement is not frequent (Lukas et al., 2009). Intake prediction with mathematical models is often the only alternative to direct DMI measurement (Viera et al., 2013; Pulina et al., 2013; Gurgel et al., 2021). Indeed, mathematical models have been used for many decades to increase animal performancea and to minimize nutrient excretion (Tedeschi et al., 2005). Since the amount of nutrients eaten by small ruminants during each physiological stage will depend on their DMI (Viera et al., 2013), it needs to be predicted accurately to achieve high performances, to maintain the animals healthy and to limit feeding costs.

The complexity in factors affecting DMI is vast, making the development and validation of prediction models very complex. Indeed, most prediction models consider only few of the variables affecting DMI in ruminants even in dairy cattle, where the majority of studies were caried out.

In sheep, Pulina et al. (1996) developed on of the most used prediction models for estimating dry matter intake for sheep reared indoors. The model for adult ewes considers as predictors body weight, milk yield, and average daily gains, which in adult animals is an estimator of variations in body reserves. The prediction is then adjusted during pregnancy, to account for the effect of days in pregnancy and litter size, with discrete adjustment factors. In a more recent study, Gallo and Tedeschi (2019) modified the discrete adjustment factors of Pulina et al. (1996), making them continuous. However, these two studies considered in the prediction models only animal factors, without accounting for factors associated to the diets. Serra (1998; reported in Pulina et al.,2013) developed a prediction model for lactating eweswhich included a dietary associated predictor. Unfortunately, all these models have not been evaluated and compared with independent data. Therefore, the objectives of this study were to evaluate and compare these DMI empirical prediction models for sheep by using the data collected in this thesis on Sarda dairy ewes during the transition phase between late pregnancy and early lactation.

4.2 MATERIAL AND METHODS

The data used for the evaluation of the model originated from the study conducted on Sarda ewes and described in Chapter 2 of this thesis. The ewes were monitored during the last 30 days of pregnancy and the first 28 days of lactation. The experiment was carried out in the experimental barn of the University of Sassari, “Azienda didattico sperimentale Mauro Deidda” in Ottava, Sassari 40°46'24.2"N 8°29'31.2"E , from December 2021 to April 2022.

4.2.1 Animal Data

Late pregnancy phase

Starting 14 days before the beginning of the feeding treatments, 28 pregnant ewes received a common diet composed of oat dehydrated hay with a supplementation of 350 g/d of whole corn grains and 140 g/d of soybean meal, on a DM basis. At 120 days in pregnancy, the ewes were divided in two groups, homogeneous for body weight (BW, mean±SD; 54±8.7), BCS, and litter number (1.24), and assigned to one of the two experimental diets, fed until parturition. One diet (OH) was made by dehydrated chopped oat hay (DM basis: 9.6% CP, 65.6% NDF, 38.5% ADF, 4.9% ADL), fed ad libitum, and a fixed amount of concentrate (350 g/d of whole corn grains and 140 g/d of soybean meal, DM basis); the other diet (AAM) was made by dehydrated chopped alfalfa hay of medium quality (DM basis: 14.8% CP, 53.3% NDF, 43.8% ADF, 9.8% ADL), fed ad libitum, and a fixed amount of concentrate (440 g/d of whole corn grains, DM basis). The concentrates were used to make the diets isoproteic and to meet the protein requirement during this physiological phase. The ewes were housed in a single pen and fed with the ®Biocontrol (Norway) CRFI manger system, which allows the measurement of the daily individual DMI in animals kept in the same pen.

Early lactation phase

After lambing, only 25 lactating ewes were retained for the second part of the experiment. Three ewes were excluded from the trial, one was not able to synthesize milk and other two had a teats orifice edema. During the lactation phase, all 25 ewes were fed ad libitum the same alfalfa dehydrated chopped hay previously used during pregnancy for the AAM diet and a fixed amount of concentrate (440 g/d of whole corn grain + 135 g/d of soybean meal, DM basis) for 28 days. All ewes were housed in the same large pen and fed with the ®Biocontrol manger system, as previously described during the pregnancy stage. Individual BW was measured daily using a precision scale of ®Biocontrol, and the ewes were weighed each morning at the exit of the milking parlor. Milk yield (MY) was recorder twice a day, at 7:00 and 18:00 respectively allowing a milking interval of 11 hours, while milk was sampled weekly for milk quality analysis.

4.2.2 Empirical models evaluated

Empirical models to predict DMI during pregnancy

Using the data collected from the experiment, two DMI prediction models were evaluated.

Model 1 (Pulina et al. 1996, reported in Pulina et al., 2013)

$DMI = (-0.545 + 0.095 \times FBW0.75 + 0.005 \times ADG) \times K$

where FBW = full metabolic weight, ADG = average daily gain, K= adjustment factor for pregnancy in weeks (BW of the litter > 4.0 kg = K is 0.82, 0.90, 0.96 for wks 1, 2-3, 4-5, respectively; BW of the litter < 4.0 kg = K is 0.88, 0.93, 0.97 for wks 1, 2-3, 4-5, respectively).

Model 2 (Gallo and Tedeschi, 2021)

Model 2 modified the discrete adjustment factors of Pulina et al. (1996) introducing an asymmetrical nonlinear function fDMI to convert these discrete adjustment factors into a continuous adjustment factor as represented by the Eq(5) as follows:

$$f_{DMI} = a + b \times \left(2x\ e\ x \begin{pmatrix} Ln\{Exp[(t + d/2)/e] + Exp[c/e]\} - \\ Ln\{Exp[(c + d/2)/e] + Exp[t/e]\} \end{pmatrix} + d \right) / (2xd)$$

$$\begin{cases} \begin{cases} a = 0.97,\ b = 1 - a,\ c = -42,\ d = 0.04,\ e = -1.3\ t \le -35 \\ a = 0.93,\ b = 0.97 - a,\ c = -28,\ d = 0.04,\ e = -1.3\ t \le -21 \\ a = 0.88,\ b = 0.93 - a,\ c = -14,\ d = 0.04,\ e = -1.3\ t \le -7 \\ a = \frac{1}{1 + ca},\ b = 0.88,\ c = 0,\ d = 0.04,\ e = -1.3\ t = 0 \end{cases} & \text{Litter weight } \le 4 \text{ kg} \\ \begin{cases} a = 0.96,\ b = 1 - a,\ c = -42,\ d = 0.04,\ e = -1.3\ t \le -35 \\ a = 0.90,\ b = 0.96 - a,\ c = -28,\ d = 0.04,\ e = -1.3\ t \le -21 \\ a = 0.82,\ b = 0.90 - a,\ c = -14,\ d = 0.04,\ e = -1.3\ t \le -7 \\ a = \frac{1}{1 + ca},\ b = 0.82,\ c = 0,\ d = 0.04,\ e = -1.3\ t = 0 \end{cases} & \text{Litter weight } > 4 \text{ kg} \end{cases}$$

where a, b, c, d, e, and f are parameters of the asymmetrical nonlinear function; ca a parameter for lactating ewe (default values are 0.52 and 0.71); Exp the exponential function; f DMI the adjustment factor for gestating ewe's potential dry matter intake; Ln the natural logarithmic function; and t is time (negative for pregnancy in which zero is at lambing) in days.

Considering this the new model for DMI prediction is:

$$DMI = ((-0.545 + 0.095 \text{ x } FBW0.75 + 0.005 \text{ x } ADG) \text{ x } K) \text{ x } fDMI$$

Empirical models to predict DMI during lactation

The data obtained from the experiment were used to evaluate four different DMI prediction models developed for lactating ewes. The data used consist of two groups of ewes, which used different diets during pregnancy but the same diet during the lactation and will be called, for the lactation stage, OHp and AAMp, to highlight their different diet during pregnancy. Model 1 (Pulina et al., 1996; cited by Pulina et al., 2013) considers only animal predictors, while Model 3 (Serra, 1998; cited by Pulina et al., 2013) also considers a diet predictor. Model 2 and Model 4 were obtained applying to Models 1 and 3, respectively, the corrections factors proposed by NRC (2007) for ewes in early lactation, as modified by Gallo and Tedeschi (2021).

Model 1 (Pulina et al., 1996)

$DMI = -0.545 + 0.095 \times BW0.75 + 0.65 \times MY \times (0.25 + 0.085 \times Fat + 0.035 \times Prot) + 0.0025 \times BWchange$

Model 2 (Gallo and Tedeschi, 2021)

DMI = (−0.545 + 0.095 × BW0.75 + 0.65 × MY × (0.25 + 0.085 × Fat + 0.035 × Prot) +0.0025× BWchange) × fDMI

Model 3 (Serra et al., 1998)

DMI = 0.0214 × BW + 0.319 × (MY × (0.25 + 0.085 × Fat +0.035 × Prot)) + 0.0373 × CPdiet

Model 4 (Serra et al., 1998 with correction factor of Gallo and Tedeschi, 2021)

DMI = (0.0214 × BW + 0.319 × (MY×(0.25 + 0.085 × Fat +0.035 × Prot) + 0.0373 × CPdiet) × fDMI

where DMI, kg/d; MY = milk yield, kg/d; Fat and Prot = milk fat and protein in %; BWchange = g/d; CPdiet = dietary CP, % DM;

$$f_{DMI} = \frac{1 + ca\ x\left(\frac{t}{cc}\right)^{1.4}\ x\ Exp\left(1.4x\left(1 - \frac{t}{cc}\right)\right)}{1 + ca}$$

where ca is a breed parameter that depends on the number of suckling lambs; cc the time at peak milk, days; Exp the exponential function; f DMI the adjustment factor for lactating ewes' potential dry matter intake; and t is the days in milk (positive values after parturition), d. The ca parameter in equation 6 depends on the number of suckling lambs and the breed. For Merino type breeds, it is 0.52 for single or 0.71 for double lambs; and for the meat-type breed, it is 0.66 for single or 0.88 for double lambs. The cc parameter is assumed fixed at 28 days (peak of lactation), but it is likely that this value changes with different breeds, the plane of nutrition, and environmental and management conditions.

4.2.3 Model evaluation

Observed (O) and predicted (P) DMI were compared by using the statistics reported by the Model Evaluation System, version 3.2.4 (https://nutritionmodels.com/mes.html) (Tedeschi, 2006) for all pregnant ewes (n = 28), as well as lactating ewes (n = 25). The following statistics were used to ensure precision and accuracy of the models: coefficient of determination (r2), which is an estimator of precision, bias correction factor (Cb, varies from 0 to 1), which indicates how far the regression line deviates from the slope of equivalence (Y=X) and is an estimate of accuracy, concordance correlation coefficient (CCC, varying from –1 to 1), which is the product of r × Cb and simultaneously account for accuracy and precision, the mean square error of prediction (MSEP) and the root mean square error of prediction (RMSEP)

4.3 RESULTS

4.3.1 Evaluation of pregnancy models

Table 3 shows the summary statistics of the evaluation of the prediction models of DMI applied during late pregnancy. The average pregnant ewe DMI was 1.43 kg/d and 1.16 kg/d for AAM and OH diets, respectively.

Regarding the pregnant ewes of the AAM group, Model 1 had lower accuracy and precision than Model 2 (r2: 0.07 vs 0.34; Cb: 0.25 vs 0.24; CCC: 0.07 vs 0.14, and RMSEP: 0.24 vs 0.36 kg/d, for Models 1 and 2, respectively; Table 3). Both Models underestimated the observed DMI (Model 1 P-O= -0.20 kg/d; P<0.01; Model 2 P-O= -0.24; P<0.01).

Model 1 had lower accuracy and precision than the Model 2 (r2: 0.17 vs 0.42; Cb: 0.50 vs 0.96 CCC: 0.21 vs. 0.63, and RMSEP: 0.15 vs 0.11 kg/d for Models 1 and 2, respectively; Table 3) also when used for gestating ewes of the OH group. Model 1 overestimate the observed DMI (P-O= 0.10 kg/d; P<0.01), while the mean bias was very low for Model 2 (P-O= -0.03 kg/d; P>0.1).

Overall, only Model 2 applied to the OH diet gave a satisfactory prediction (P=0.048). Model 1 and 2 for gestating ewes in AAM group and Model 1 for gestating ewes in OH group were not able to predict adequately the voluntary DMI in this physiological phase (Figure 1 and 2).

4.3.2 Evaluation of early lactation models

Table 4 shows the results of the prediction of DMI using Model 1, 2, 3 and 4 for the predicted DMI, respectively. Regarding the ewes fed AAM diets during pregnancy, DMI in early lactation was overestimated by all the 4 models: Model 1 overestimated DMI by 0.71 kg/d (P=0.07), Model 2 by 0.43 kg/d (P<0.01), Model 3 by 0.42 kg/d (P<0.01) and Model 4 by 0.16 kg/d (P>0.1). For the lactating ewes of the AAM group, the regressions of predicted on observed values had r2= 0.62, 0.81, 0.56, and 0.82 for Models 1, 2, 3, and 4, respectively. The Cb was 0.18, 0.53, 0.19, 0.86 for Models 1, 2, 3 and 4, respectively. The CCC was 0.14, 0.48, 0.14, and 0.79 for Models 1, 2, 3, and 4, respectively. The RMSEP was 0.72, 0.48, 0.46, 0.20 for Models 1, 2, 3 and 4, respectively.

Regarding the ewes fed the OH diet during pregnancy, DMI in early lactation was also overestimated by all the 4 models: Model 1 overestimated DMI by 0.66 kg/d (P=0.01), Model 2 by 0.40 kg/d (P=0.03), Model 3 by 0.43 kg/d (P<0.01) and Model 4 by 0.19 kg/d (P>0.1). For the lactating ewes of OH group, the regressions of predicted on observed values had r2= 0.80, 0.92, 0.68, and 0.12 for Models 1, 2, 3, and 4, respectively. The Cb was 0.19, 0.57, 0.16, 0.83 for Models 1, 2, 3 and 4, respectively. The CCC was 0.17, 0.55, 0.13, and 0.79 for Models 1, 2, 3, and 4, respectively. The RMSEP was 0.67, 0.42, 0.49, 0.20 for Models 1, 2, 3 and 4, respectively. Only Model 2 and 4 applied to the AAM and OH diet gave a satisfactory prediction compared with Model 1 ($P<0.001$) and Model 3 ($P<0.001$). Comparing Model 2 and Model 4, Model 4 was the most precise in predicting DMI, with a r2=0.82 in AAM group and r2=0.91 in OH group, respectively.

4.4 DISCUSSION

The findings of this study suggest that the adjustments proposed by Gallo and Tedeschi gestating ewes improved the prediction only for the OH diet (gestation Model 2). The results suggested that for the late pregnancy period, the models tested, which included only animal associated predictors were not able to predict with precision and accuracy the voluntary dry matter intake (VDMI) when the diet was based on high levels of supplementation of forages. We already know that VDMI is controlled by the physiological requirements of the animals but also by the filling effect of the diet (Conrad, 1966; Ingvartsen et al., 1992; Tedeschi et al., 2013; Tedeschi and Fox, 2018; Cannas, 2010). Thus, in addition to animal factor, it would be necessary to consider diet quality factors. Dietary CP and NDF could be two important predictors to use, since CP is important for covering N requirements of rumen microorganisms and NDF is the major factor affecting the rumen fill intake (Van Soest, 1994 and Mertens, 1994). It is evident from Figure 1 that for the AAM group both models underestimated the DMI during late pregnancy, while for OH group (Figure 2) they overestimated it. This could be explained by the differences in forage quality, since the physiological status was the same for both groups. The OH diet was based on oat forage with a high level of NDF (65.6 % of NDF), that could have created a high rumen fill on the animals, while Models 1 and 2 did not consider this limiting factor. On the other hand, AAM diet was based on high quality alfalfa hay, with a low level of NDF (53.3% of NDF) but also a higher percentage of CP (14.8% of CP), and thus a probably higher digestibility. This could have increased the DMI of the animals, both for the low amount of indigestible fiber and the better palatability of the forage. The effect of the pregnancy on VDMI it is visible for the two group as they approached the lambing day. In fact, there was a difference on the drop of the DMI between the groups. For AAM the drop in DMI started from the 5th day before lambing, while for the OH group started only the 3rd day before lambing, because of the pressure of the fetus on the rumen and, possibly, the changing position of the same in the uterus. The limiting factor for OH diet was certainly the NDF content of the forage, which limited the DMI of the ewes and didn’t allow them to cover all their energetic requirements. The ewes of OH diet for that reason arrived at the day of lambing with a more negative energetic balance compared with the one of AAM group (Chapter 2 of this thesis). For lactating ewes, the findings of this study suggest that the adjustments (fDMI) proposed by Gallo and Tedeschi (2021) improved the predictions of DMI compared to the models of Pulina et al. (1996) and Serra et al. (1998) (lactation Models 2 and 4 vs. Models 1 and 3). However, as shown in Figures 3 and 4, all models overestimated the observed DMI for both diets. This may be because the models that we tested did not consider the NDF content of the diet and its effects on the DMI in terms of fill, even though this is a well-known factor limiting the intake of ruminants (Van Soest, 1994 and Mertens, 1994). As Gallo and Tedeschi (2021) also reported, Model 1 was developed considering diets that are predominantly pelleted and not fed as whole forage, partially explaining the overestimation of all the models. The comparison among the models (Table 4) showed that Model 4 had the highest accuracy and precision compared to Model 1, 2 and 3, respectively. In fact, Model 4 is the result of Model 3 multiplied by the fDMI proposed by Gallo and Tedeschi (2021). This means that the presence in the Model 4 of a continuous adjusting factor for early lactation and a predictor associated to the quality of the diet (CP of the diet), significantly improved the accuracy of that model in predicting DMI in early lactation. Again, as previously discussed for the late pregnancy period, also in early lactation it is important to consider not only animal factors but also factors associated to diet quality to improve DMI predictions. Overall, Model 4, which is the combination of animal-based predictors, a diet quality predictor, and the continuous adjusted factor (fDMI) for early lactation proposed by Gallo and Tedeschi (2021) was the only model able to predict adequately the observed data.

4.5 CONCLUSIONS

In conclusion, the comparison of various models of DMI prediction in late pregnancy and early lactation highlighted the need of considering, in the prediction models, the peculiar pattern of DMI during the transition stage and also predictors associated to dietary factors (e.g., CP and NDF of the diet). It appears that to further develop prediction models for the transition stage is necessary to accumulate more intake data to be collected specifically in this stage.

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4.7 TABLES AND FIGURES

Table 1. Ingredients of experimental diets of ewes in late gestation and early lactation, OH = oat group and AAM= alfalfa group

	Pregnancy				Lactation	
	OH		AAM		AAM	
Feed	AF[a]	DM[b]	AF[a]	DM[b]	AF[a]	DM[b]
Forage, kg	Ad libitum	Ad libitum	Ad libitum	Ad libitum	Ad libitum	Ad libitum
Soybean meal, kg	0.150	0.140	-	-	0.150	0.140
Whole corn grain, kg	0.400	0.350	0.400	0.350	0.500	0.440

[a]AF =as fed
[b]DM = dry matter

Table 2. Chemical composition of forages and concentrates fed and mineral integration in late gestation and early lactation

	Pregnancy		Lactation		
Chemical composition	Dehydrated Oat	Dehydrated Alfalfa	Dehydrated Alfalfa	Whole Corn grain	Soybean meal
Dry matter, % as fed	88.6	89.0	89.0	88.0	90.0
CP, % DM	9.6	14.8	14.8	9.8	49.9
NDF, %DM	65.6	53.3	53.3	10.0	14.9
ADF, % DM	38.5	43.8	43.8	-	-
ADL, % DM	4.9	9.8	9.8	0.2	0.3
Ash, % DM	8.8	9.0	9.0	1.6	7.2
EE, % DM	2.2	1.8	1.8	4.3	1.6
Ca, % DM	0.3	1.2	1.2	0.04	0.3
P, % DM	0.2	0.2	0.2	0.3	0.7
Mineral and vitamins*, % DM	3.3	3.3	2.7		

DM=dry matter

Mineral composition*: Ca = 146 g, P = 30 g, Mg = 38 g, Na = 130 g, I = 50 mg, Mn = 1,800 mg, Se = 8.3 mg, Zn = 2.880 mg, vitamin A = 500,000 U.I., vitamin D3 = 50.000 U.I., vitamin E = 1244 U.I., vitamin B1 = 15 mg, vitamin B2 = 18 mg, vitamin B6 = 9 mg, vitamin B12 = 52.7 mg

Table 3. Statistics used to evaluate the prediction models of dry matter intake (DMI) in late pregnancy.

Model	Group	Observed mean	Predicted mean	r^2	Cb	CCC	RMSEP	*P*-value
1	AAM	1.43	1.23	0.07	0.25	0.07	0.24	<0.001
2	AAM	1.43	1.09	0.34	0.24	0.14	0.36	
1	OH	1.16	1.26	0.17	0.50	0.21	0.15	0.048
2	OH	1.16	1.13	0.42	0.96	0.63	0.11	

AAM=alfalfa hay DMI during pregnancy; OH=oat hay DMI during pregnancy, r^2= coefficient of determination, CCC=concordance correlation coefficient, Cb=bias correction factor, MSE=mean square error, RMSEP=root mean square error of prediction

Table 4. Statistics used to evaluate the prediction models of dry matter intake (DMI) in early lactation. The data used consist of two groups of ewes, which used different diets during pregnancy but the same diet during the lactation and will be called, for the lactation stage, OHp and AAMp, to highlight their different diet during pregnancy

		Observed	Predicted					
Model	Group	mean	mean	r^2	Cb	CCC	MSEP	RMSEP
1	AAMp	1.99	2.70	0.62	0.18	0.14	0.54	0.72
2	AAMp	1.99	2.42	0.81	0.53	0.48	0.24	0.48
3	AAMp	1.99	2.41	0.56	0.19	0.14	0.22	0.46
4	AAMp	1.99	2.15	0.82	0.86	0.79	0.04	0.20
1	OHp	1.87	2.53	0.80	0.19	0.17	0.46	0.67
2	OHp	1.87	2.27	0.92	0.57	0.55	0.18	0.42
3	OHp	1.87	2.31	0.68	0.16	0.13	0.24	0.49
4	OHp	1.87	2.06	0.91	0.83	0.79	0.04	0.20

AAM=alfalfa hay DMI during lactation; OH=oat hay DMI during lactation, r^2= coefficient of determination, CCC=concordance correlation coefficient, Cb=bias correction factor, MSE= mean square error, RMSEP=root mean square error of prediction,

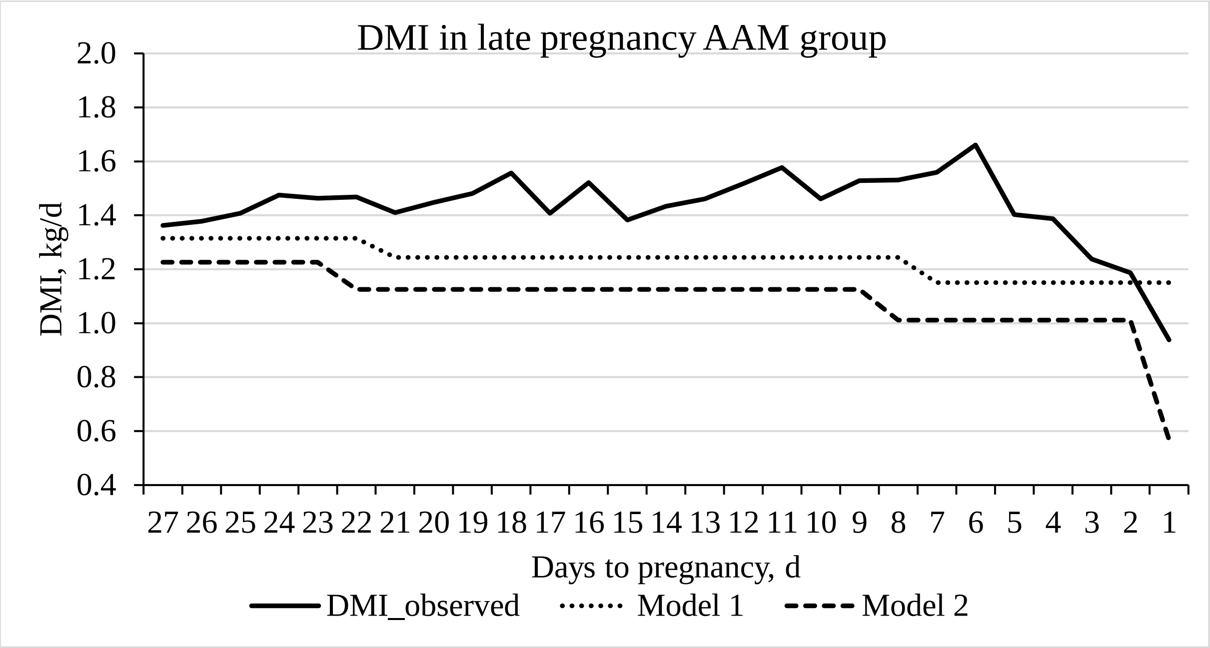

Figure 1. Comparison of observed and predicted DMI during late pregnancy in the AAM group. Model 1=Pulina et al., 1996 reported by Pulina et al., 2013. Model 2=Gallo and Tedeschi., 2021.

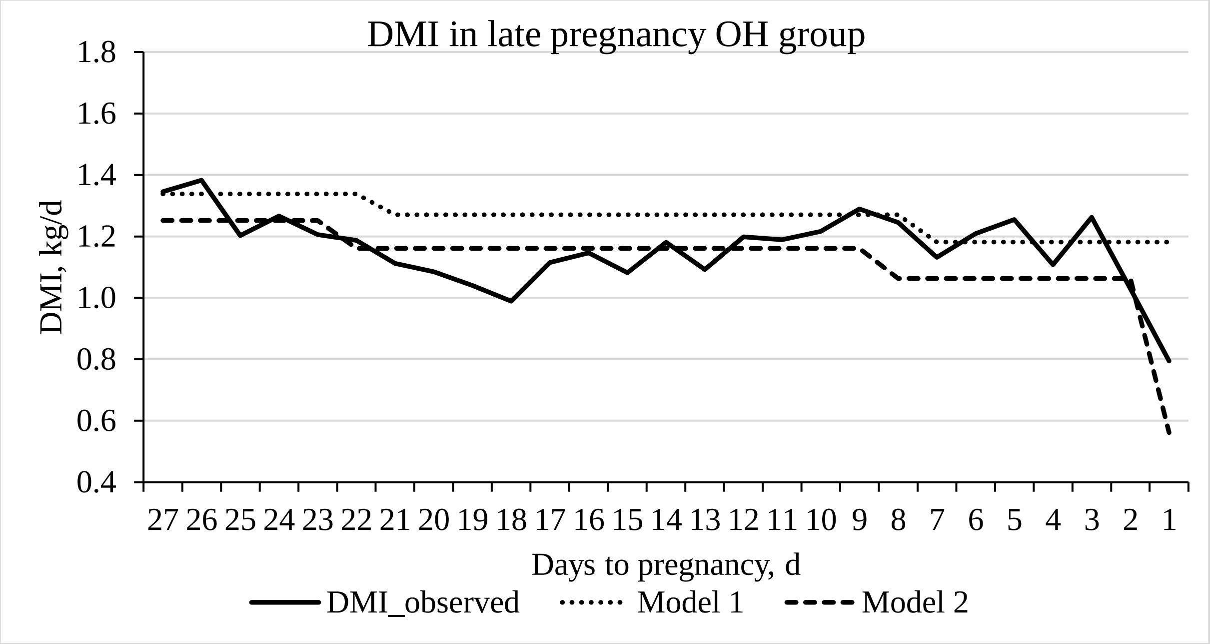

Figure 2. Comparison of observed and predicted DMI during late pregnancy in the OH group. Model 1=Pulina et al., 1996 reported by Pulina et al., 2013. Model 2= Gallo and Tedeschi, 2021.

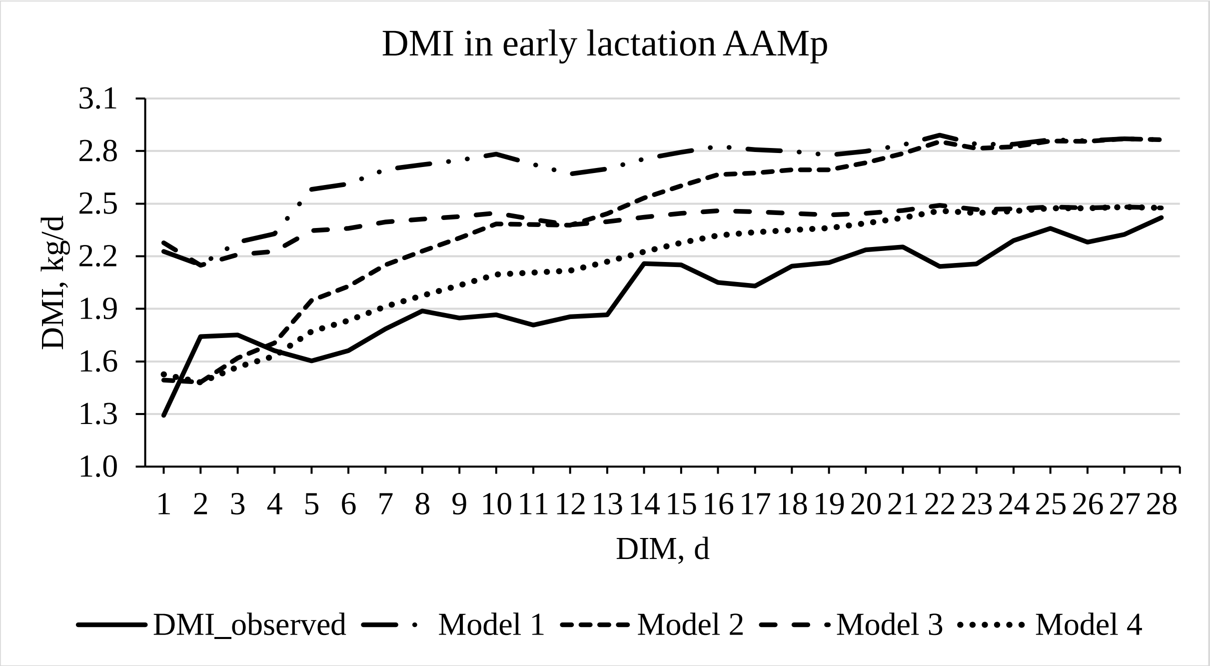

Figure 3. Comparison of observed and predicted DMI during early lactation. The data used consist of two groups of ewes, which used different diets during pregnancy but the same diet during the lactation and will be called, for the lactation stage, OHp and AAMp, to highlight their different diet during pregnancy**.** Model 1=Pulina et al., 1996 reported by Pulina et al., 2013; Model 2=Gallo and Tedeschi., 2021; Model 3= Serra, 1998; Model 4= Serra, 1998 adjusted with the correction facto of Gallo and Tedeschi, 2021.

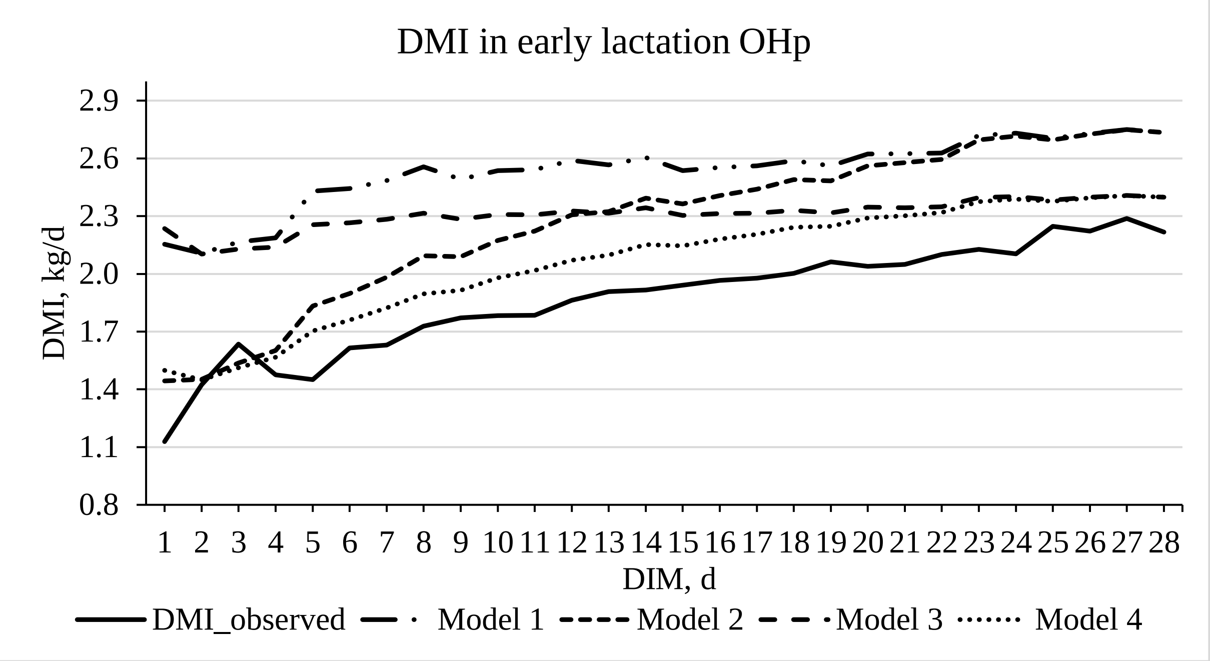

Figure 4. Comparison of observed and predicted DMI during early lactation. The data used consist of two groups of ewes, which used different diets during pregnancy but the same diet during the lactation and will be called, for the lactation stage, OHp and AAMp, to highlight their different diet during pregnancy**.** Model 1=Pulina et al., 1996 reported by Pulina et al., 2013; Model 2=Gallo and Tedeschi., 2021; Model 3= Serra, 1998; Model 4= Serra, 1998 adjusted with the correction facto of Gallo and Tedeschi, 2021**.**

5 CHAPTER 5

General conclusions and implications

Based on the review of the literature (Chapter 1), it is clear the major importance of forage quality and fiber in the nutrition of small ruminants, having the goal to ameliorate their production capacity and longevity. Fiber is the main component of animal feeds and this review highlighted the factors affecting voluntary fiber intake, voluntary dry matter intake and the effect of forage sources on their rumen mean retention time. However, most of the studies were carried out of the transition stage, i.e. late pregnancy and early lactation, especially in the case of small ruminants. The same applies for the models developed to predict intake, which rarely account for the peculiar behavior of intake during the transition stage. In addition, for these species there is also limited information on the effects of forage quality and their fiber quality on lactation persistency. In the case of sheep, in which this thesis was focused, most of the studies were conducted in meat or wool sheep breeds, which have a short lactation, while much less research focused on dairy ewes, which have a much longer lactation and thus higher milk production persistency. Considering that worldwide there in an increasing interest in dairy milk ewes and an increasing demand of their products, there is a great need to improve the knowledge on the effects of forages quality on dairy ewes. Thus, during this thesis two experiments were carried out on dairy ewes in the transition stage and in mid lactation. In addition, available prediction models for dairy sheep were evaluated.

The first study (Chapter 2) focused on the transition stage, comparing two forages sources of different quality during late pregnancy end their residual effects in early lactation. The utilization of the forage with the highest quality (dehydrated chopped alfalfa hay) during the late pregnancy period had a positive impact on voluntary DMI, hay intake, NDFI and energy balance of the ewes when compared to a forage of lower quality (dehydrated chopped oat hay). Our findings confirmed that the reference values of NDFI of 1.0-1.1 in % of BW proposed by Cannas et al. (2016) suggested to maximize energy intake during pregnancy. There was not a clear negative effect of the diet supplied during the pregnancy phase on the subsequent lactation performances in terms of DMI, NDFI and milk yield, when the ewes were fed during early lactation a diet made by the same alfalfa hay using during pregnancy. Thus, it seemed that the utilization of forages of high quality during early lactation partially compensated the undernutrition at which ewes were exposed during the last month of pregnancy when fed the low quality OH forage. Thus, this study suggested that the utilization of high-quality forages must to be considered as a pivotal point in the nutritional management of the transition from pregnancy to lactation.

The second experiment (Chapter 3), clear showed that forage quality can have dramatic effect on milk production persistency even during mid lactation, despite the fact that in this stage milk production was lower than in the first part of the lactation. Three different forages were tested, feeding them ad libitum and supplying a fixed dose of concentrates. The forage with the highest quality (high-quality alfalfa hay) induced in the highest DMI, milk production, milk protein and milk fat yield, while that of lowest quality (oat hay) had the lowest values for this production variables.

Finally, various models to predict DMI were evaluated (Chapter 4) by using the data collected in the experiment described in Chapter 2. The evaluation highlighted the need of considering in the prediction models the peculiar pattern of DMI during the transition stage and also predictors associated to dietary factors (e.g. CP and NDF of the diet), to account for the dietary effects on intake. It appears that current prediction models have many limitations and more research is needed to improve them.