NUTRIENT INTAKE AND DIGESTIBILITY AND RUMINAL RATE PASSAGE IN PREGNANT EWES

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ABSTRACT

This research was carried out to evaluate the effects of pregnancy, number of fetuses and feed restriction on nutrients intake and digestibility and on ruminal rate passage. Twenty-four ewes were used at 90, 110 and 130 days of pregnancy, and distributed among treatments according to the number of fetuses. They received balanced diet or restricted diet at 15% of energy and protein requirement. Ewes with two fetuses on feed restriction had more dry matter intake than those with one fetus. At 130 day, the highest dry matter and neutral detergent fiber intake occurred. Twin-pregnant ewes showed the highest nutrient digestibility, except for fiber fraction. Feed restriction caused smaller dry matter intake. Ruminal rate passage increased in late pregnancy.

KEYWORDS: feed management; number of fetuses; prepartum; sheep.

CONSUMO, DIGESTIBILIDADE E TAXA DE PASSAGEM RUMINAL EM OVELHAS GESTANTES

RESUMO

O objetivo deste estudo foi avaliar a influência da gestação, número de fetos e restrição nutricional no consumo e digestibilidade de alguns componentes químicos e a taxa de passagem ruminal. Foram utilizadas 24 ovelhas aos 90, 110 e 130 dias de gestação, divididas entre os tratamentos em função do número de fetos. Os animais receberam dietas balanceadas ou restritas em 15% nos requisitos de energia e proteína. Ovelhas com dois fetos submetidas à restrição nutricional consumiram mais matéria seca que aquelas com um feto. Aos 130 dias de gestação, houve maior consumo de matéria seca e fibra em detergente neutro. Ovelhas de gestação dupla apresentam maior digestibilidade dos nutrientes, exceto nas frações fibrosas. A restrição alimentar acarretou menor ingestão de matéria seca. A taxa de passagem ruminal aumenta ao final da gestação.

PALAVRAS-CHAVE: manejo alimentar; número de fetos; ovinos; pré-parto.

INTRODUCTION

At the beginning of the pregnancy period of the ewe, fetal growth is small and determined by genetic patterns of the species. During the final third, however, major fetal growth occurs, directly influenced by maternal nutrition (MACEDO JUNIOR et al., 2010). For sheep, up to 90% of newborns weight is obtained during the last two months of pregnancy. MACEDO JUNIOR et. al. (2010) stated that females with nutritional deficiency, especially in the final third, may have offspring with low birth weight, increasing the mortality rate of newborns in the production system.

At the end of pregnancy, besides the increase in nutritional requirements caused by the rapid growth of fetuses, two other factors negatively contribute to energy balance/ reduction of the energy use from food and less appetite due to the decreased volume of the gastrointestinal tract promoted by the largest space occupied by the fetus and annexes. However, the consumption reduction is proportionately smaller than the decrease in rumen volume, probably due to the increased passage rate (MACEDO JUNIOR et al., 2010; BENEVIDES et al., 2011). Several mechanisms may explain the causes of increased passage rate with the progress of gestation. Regarding the physical aspect, the pressure exerted by the uterus in the rumen, reticulum, omasum and abomasum may stimulate motility and increase in intra-ruminal pressure. It can increase the sensitivity of rumen receptors resulting in increased propulsive activity during late gestation (MACEDO JUNIOR et al., 2010; BENEVIDES et al., 2011). Furthermore, circulating concentrations of estrogen, progesterone and prolactin could also be responsible for changes in the passage rate of digesta (URESTE, 2001). According to MACEDO JUNIOR et al. (2010) and BENEVIDES et al. (2011), in late pregnancy, greater amount of grains is included in the diet of sheep, which may also contribute to the increase in passage rate.

Despite the increased nutritional requirements of pregnant ewes, high energy diets can cause fat accumulation in the body fat and difficulties in childbirth. On the other hand, energy-deficient diets cause problems during gestation in sheep, with consequent production of fragile lambs weighing below average. Accordingly, maternal nutrition during prenatal period can be a determining factor in the ability of the matrix sheep to produce milk during lactation (COSTA et al., 2003; PEDROSO et al., 2004; LUO et al., 2004a; LUO et al., 2004b; LUO et al., 2004c; LUO et al., 2004d; LUO et al., 2004e; LUO et al., 2004f; PODLESKIS et al., 2005; MARIZ et al., 2007; TURINO et al., 2007; MACEDO JUNIOR et al., 2009; MACEDO JUNIOR et al., 2010; BENEVIDES et al., 2011).

This study was conducted to evaluate nutrients intake and digestibility in pregnant Santa Ines ewes with one or two fetuses, submitted to food restriction at 90, 110 and 130 days, and to assess the solids passage rate.

MATERIAL AND METHODS

The experiment was conducted in the Department of Animal Science, Faculty of Veterinary Medicine, Federal University of Minas Gerais, in Belo Horizonte.

We used 24 Santa Ines sheep, which were vaccinated and received vermifuge. We confirmed pregnancy and fetal quantification at 60 days of gestation by ultrasonography. We divided the animals into two groups according to the number of fetuses (one or two). Subsequently, in each group, we randomly divided the animals into the following feeding management groups (balanced diet or restricted): pregnant ewes with one fetus and balanced diet; pregnant ewes with one fetus and restricted diet, pregnant ewes with two fetuses and balanced diet; and pregnant ewes with two fetuses and restricted diet. The sheep were housed individually in metabolic cages with slatted floor and suitable devices for collecting feces and urine separately.

The experiments were conducted from the 85th to the 90th, from the 105th to the 110th, and from the 125th to the 130th days of gestation, characterizing the phases 90, 110 and 130 days, respectively. At each stage of evaluation, the experiment lasted 20 days, 15 for adaptation and five for sample collection of samples of diet offered, leftovers, feces and urine. We weighed the animals at the beginning of the adaptation period and used the weight to calculate the percentage of consumption in live weight and grams per kilogram of metabolic weight ($kg^{0.75}$).

We formulated the diets according to the recommendations of the NRC (1985), to meet the requirements for total digestible nutrients (TDN) and crude protein (CP), for maintenance and pregnancy, and supplied it twice a day. The animals fed balanced diet received ration amounts adjusted daily, depending on leftovers, maintaining always 10% above what was consumed, while the group fed restricted diet received the amount of energy and protein recommended by the NRC (1985) reduced by 15%. The ration consisted of chopped Tifton 85 hay (C*ynodon* spp.), concentrate composed of 81% ground corn, 18% soybean meal and 1% limestone (percentage in dry matter). At 120 days of gestation, diets were adjusted following the recommendations of NRC (1985). The proximate composition and nutritive value of the experimental diets are presented in Table 1. Water and mineral salt (Vacci-phós, Vaccinar®) were provided *ad libitum* in a proper place.

Table 1 – Centesimal and chemical composition of the experimental diets

Ingredients	Up to 120 days of gestation			
	1 fetus restricted diet	1 fetus unrestricted diet	2 fetuses restricted diet	2 fetuses unrestricted diet
Corn meal	8.73	26.35	28.48	49.81
Soybean meal	1.93	4.55%	5.17%	8.09
Tifton hay	89.25	68.82	66.04	41.63
Limestone	0.11	0.28	0.31	0.47
Crude protein	7.93	9.32	9.60	11.19
TDN[1]	47.38	55.74	56.86	66.89
NDF[2]	70.55	56.47	54.55	37.68
	120 to 150 days of gestation			
Ingredients	1 fetus restricted diet	1 fetus unrestricted diet	2 fetuses restricted diet	2 fetuses unrestricted diet
Corn meal	11.90	30.57	26.09	43.69
Soybean meal	5.27	8.50	5.79	9.29
Tifton hay	82.65	60.53	67.78	46.41
Limestone	0.18	0.40	0.34	0.61
Crude protein	9.27	10.91	9.78	11.50
TDN[1]	50.00	59.00	56.08	64.76
NDF[2]	66.02	50.73	55.74	41.03

[1]Total digestible nutrients (estimated by the program for diet formulation); [2]neutral detergent fiber.

Samples of feed and leftovers were collected before the first feeding, weighed and stored in plastic bags. Feces were collected and weighed, withdrawing a 20% sample of the total production. The material was packed in individual plastic bags and stored in a cold (-15°C) chamber. At the end of the experiment, samples were defrosted under ambient conditions for 14 hours, sieved through a coarse mesh, homogenized and weighed. Subsequently, they were placed in an oven with forced ventilation for 72 hours at 60°C for determination of pre-dry matter. Samples of feed, leftovers and feces were ground in a mill equipped with 1 mm sieve and packed in plastic containers.

We placed 100 ml of sulfuric acid (H_2SO_4.2N) in the buckets for urine collection, on the day before each collection to avoid nitrogen loss by volatilization. The total volume of liquid was weighed and measured, removing a 20% aliquot from the total volume collected daily. The aliquot was filtered through gauze and placed in plastic flasks in a cold chamber (-15°C). We determined urine density by means of a refractometer and measured water and salt consumption by the difference between the quantity supplied and the leftovers. We placed the salt remains in an oven at 60°C with forced ventilation for 24 hours for determination of the pre-dried matter.

Feed, leftovers and feces samples were analyzed for dry matter, ash (AOAC, 1980) and neutral detergent fiber (VAN SOEST et al., 1991). Consumption was calculated as the difference between the amount of food offered and the leftovers after 24 hours. The digestibility was calculated using the formula proposed by MAYNARD et al., (1984).

$$\mathrm{DA} = \frac{(\mathrm{kgcons}\times\%\mathrm{cons})(\mathrm{kgsb}\times\%\mathrm{sb})(\mathrm{kgfz}\times\%\mathrm{fz})}{(\mathrm{kgcons}\times\%\mathrm{cons})(\mathrm{kgsb}\times\%\mathrm{sb})}\times 100$$

Where:
kgcons = amount of food consumed
% cons = nutrient content of the food provided
kg sb = amount of leftovers removed
% sb = nutrient content of the leftovers
kg fz = amount of feces collected
% fz = nutrient content in the feces

The experimental groups were randomly designed, and, for the variables of intake and apparent digestibility of nutrients, animals were distributed in a (2x2) factorial design: two nutrition managements (restricted and unrestricted) and two types of gestation (single and twin). Each phase of pregnancy (90, 110 and 130 days) was evaluated separately to obtain specific responses. Thus, six replicates per treatment were obtained. We used the SNK test at 5% probability for comparison of means. We employed the following model for the analysis of consumption and digestibility at each pregnancy stage:

$Y_{ijk} = \mu + M_i + N_j + MN_{ij} + \varepsilon_{ijk}$

Where:

Y_{ijk} = observation relative to the k^{th} sheep, i^{th} feed management and j^{th} number of fetuses;

μ = population mean;

M_i = Effect of the i^{th} feed management, i= 1 and 2;

N_j = Effect of the j^{th} number of fetuses, j = 1 and 2;

MN_{ij} = Interacion of the i^{th} feed management with the j^{th} number of fetuses;

ε_{ijk} = random effect of the k^{th} sheep, i^{th} feed management and j^{th} number of fetuses;

k= 1, 2, 3, 4, 5 and 6 sheep evaluated per treatment (replication).

To evaluate the passage rate of solids, four sheep were selected from each treatment after the digestibility trial of 90 (middle third) and 130 (final third) days of pregnancy. The forage was mordanted with potassium dichromate (ÚDEN et al., 1980), provided in 10g pulse dose of chromium-mordant (small paper bags were made and placed in the animals' mouth to be swallowed), and stool samples were collected at 0, 5, 8, 11, 14, 17 , 20, 23, 26, 35, 47, 71 and 96 hours after the marker supplying. We processed the samples and analyzed dry matter (AOAC, 1980) and Cr by atomic absorption (WILLIAMS et al., 1962).

We estimated kinetic parameters of rumen transit by adjusting the curve to the fecal excretion of the marker to the time-dependent gamma-2 model (ELLIS et al., 1994):

$C_t = Z * (t - \tau) * L * \exp[-L*(t - \tau)]$, where:

Ct = fecal concentration of the marker at time "t" (ppm); t = relative time after supplying the marker (h); L = time-dependent rate parameter relative to rumen flow of particulates (h^{-1}); Z= parameter without direct biological interpretation (ppm. h); and τ = time elapsed between the application and the appearance of the marker in feces (h).

The mean retention time in the rumen-reticulum and in the gastrointestinal tract was estimated by the following equation (ELLIS et al., 1994):

$TMRR = 2/L$

$TMRT = TMRR + \tau$, where:

TMRR = mean retention time in the rumen-reticulum (h); TMRT = total mean retention time (h); L = parameter of time-dependent rate relative to the rumen flow of particles (h^{-1}); and τ = time elapsed between application and appearance of the marker in the feces (h).

RESULTS AND DISCUSSION

The dry matter intake (DMI) in kg/day, % of body weight (BW) and g/kg of metabolic body weight ($BW^{0.75}$) were influenced ($p < 0.05$) by the nutritional management (restricted and not restricted) and number of fetuses (one and two) (Table 2).The animals that received diet with restricted energy and protein had lower DMI, probably due to the greater proportion of forage offered. According to MERTENS (1987), mechanisms of physiological and physical control may act as primary limiting factors of consumption because of the energy density and the fiber contents of the diets. ZANINE & MACEDO JUNIOR et al. (2006) reported that the presence of fiber, especially of low quality, can limit dry matter intake.

There are correlations between voluntary intake and NDF content due to the relationship of the fiber with the occupation of space by the forage (MERTENS & ROTZ, 1989). Thus, if the intake is limited by the occupation of the gastrointestinal tract, food with high content of NDF have their intake restricted. Therefore, the animal consumes food until it reaches the maximum NDF intake, which happens to inhibit it, thus there is a limit of rumen degradation that determines the interruption of consumption.

The DMI (kg / day, % of BW, and g / kg $BW^{0.75}$) was higher ($p < 0.05$) for ewes carrying twin fetuses, who received a greater volume of food than ewes carrying one fetus, since the consumption recommendations were based on weight range of animals, number of fetuses and stage of pregnancy, according to NRC (1985). SOUZA JR et al. (2007) and NUNES et al. (2011) studied sheep and found higher dry matter intake in diets with lower fiber content. In the present study, animals in the unrestricted group and with two fetuses had a higher proportion of grain in the composition of the feed.

The NRC (1985) states that in ewes in the early pregnancy stage and with live weight of 50 kg the DMI should be 2.2% of body weight. In this study, the sheep had a mean weight of 53.5 kg and showed consumption similar to the recommendation by the committee.

Table 2 - Dry matter intake and apparent digestibility coefficient of restricted and unrestricted diets for Santa Inês sheep at 90 days of gestation of one or two fetuses

	Intake (Kg/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean
Unrestricted	1.34	1.62	1.48A	2.67	2.94	2.80A	70,98	80,1	75,54A	57	69	63A
Restricted	1.00	1.34	1.17B	2.16	2.46	2.31B	56,35	66,62	61,48B	56	63	59B
Mean	1.17b	1.48a		2.41b	2.69a		63,66b	73,36a		56b	65a	
CV (%)		11.3			10.6			9.0			71	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ (p <0.05) by SNK test.

The number of fetuses and the food restriction caused significant variation (p <0.05) in apparent digestibility coefficient of dry matter (ADCDM).The ADCDM was 56% for animals with simple pregnancy and 65% for those with double pregnancy. Ewes receiving unrestricted and restricted diet presented ADCDM of 63% and 59%, respectively. Possibly the greater amount of grain and greater food availability, which allows the selection of more digestible fractions, for ewes pregnant with twins and submitted to restricted diet contributed to this result, which confirms the values found for dry matter intake. MACEDO JUNIOR et al. (2009) studied pregnant Santa Ines sheep receiving increasing inclusions of neutral detergent fiber in the diet, and did not observe changes in digestibility. However, MACEDO JUNIOR et al. (2010) studied pregnant ewes (with one and two fetuses) submitted to unrestricted or restricted diets, and observed that females without restriction and with two fetuses had higher digestibility, similar to what was observed in the current study.

According to DOREAU et al. (2003), the main cause of variation in the digestibility of the diet is the time of particle retention in the rumen. Therefore, dietary restriction would lead to a reduction in the passage rate and consequent increase in digestibility. However, these authors observed different digestibility responses in animals kept under dietary restriction, but did not explain all the factors that led to these variations, especially those related to the decrease in digestibility. In the present study, the nutritional restriction did not increase digestibility. Several factors may contribute to the diversity of responses, from the level of concentrate in the diet to the adaptation mechanisms in nutrient absorption. For MACEDO JUNIOR et al. (2007), the presence of low quality fiber in the diet for sheep, especially lambs, pregnant ewes and lactating ewes may affect dry matter intake and digestibility.

We observed that ewes carrying to fetuses and submitted to unrestricted diet presented lower NDF intake (NDFI) (678.9 g / day, 1.23% BW and 33.58 g / kg BW$^{0.75}$) (Table 3). This result may be due to the greater amount of grain in the diet for these animals, and the possibility of selecting less fibrous and more digestible fibers.

Table 3 - Neutral detergent fiber intake and apparent digestibility coefficient of restricted and unrestricted diets for Santa Ines sheep at 90 days of pregnancy with one or two fetuses

	Intake (g/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean
Unrestricted	825.2Aa	678.9Bb	752.0	1.65Aa	1.23Bb	1.44	43,75Aa	33,58Bb	38,67	50	51	51
Restricted	747.4Aa	829.9Aa	788.7	1.61Aa	1.52Aa	1.57	41,97Aa	41,33Aa	41,65	58	56	57
Mean	786.3	754.4		1.63	1.38		42,86	37,46		54	53	
CV (%)		9.4			9.9			7.7			13.5	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ (p <0.05) by SNK test.

The NDF concentration in the feed is one of the factors that can limit the intake (VAN SOEST, 1994), but the results suggest that there is no physical limitation on NDFI, because the animals were at early stages of pregnancy and compression of the rumen as well as hormonal action had not started yet. Not only the NDF concentration but also its digestibility may impose limitations on the DM and energy intake, restricting the animals' productive performance (TURINO et al., 2007). In this study, NDF digestibility was not affected by the variables studied and also showed no interactions.

We observed that DMI (kg per day) was lower for sheep at 110 days of pregnancy with one fetus and submitted to restrict feeding (1.03 kg / day), because these animals present lower nutritional requirements and the diet was restricted to 15% of protein and energy. URESTE (2001) studied dairy ewes between 2 and 8 weeks before parturition, and observed average consumption during the experimental period of 2.17 kg / day. The highest values found by the author are due to the use of heavier breeds, since the consumption in relation to the metabolic weight was close to what was observed in this study. In ewes pregnant with one fetus and receiving balanced diet, the author noted that DMI of Manchega and Lacaune ewes was 80 and 88 g / kg $BW^{0.75}$, respectively, and the mean intake of these sheep was 80.64 g / kg BW $^{0.75}$.

Table 4 - Dry matter intake and apparent digestibility coefficient of restricted and unrestricted diets for Santa Ines sheep at 110 days of pregnancy with one or two fetuses

	Intake (kg/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mea	1fetu	2fetuses	Mea	1fetus	2fetuses	Mea	1fetus	2fetus	Mean
Unrestricted	1.53Aa	1.29Aa	1.40	3.03Aa	2.36Ab	2.69	80,64Aa	64,00Ab	72,32	62	70	66A
Restricted	1.03Bb	1.32Aa	1.17	2.21Ba	2.43Aa	2.32	57,72Ba	65,82Aa	61,77	56	61	58B
Mean	1.28	1.3		2.62	2.39		69,18	64,9		59b	65a	
CV (%)		17.9			19.5			18.4			10.7	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ (p <0.05) by SNK test.

The DMI in g/kg $BW^{0.75}$ for ewes with single pregnancy was lower for those undergoing nutritional restriction (57.72 g/kg $BW^{0.75}$) compared to those submitted to unrestricted diet (80.64 g/kg $BW^{0.75}$) (Table 4). In ewes with twin pregnancy, there was no difference between feeding managements. When balanced diet was offered, double pregnancy led to lower consumption in relation to metabolic weight and live weight, indicating that the expansion of the uterus began to compress the rumen, which may cause physical discomfort and reduce consumption by animals.

During pregnancy, the demand for nutrients by the fetus increases progressively as well as the volume it occupies in the abdominal cavity. These physical and metabolic changes affect voluntary food intake. The exact mechanism by which intake is inhibited due to the smaller rumen space is unknown, but it might be due to discomfort in the gastrointestinal tract (VAN SOEST, 1994). BENEVIDES et al. (2011), studied sheep with simple and twin pregnancy, under food restriction or not, and observed that the progress of gestation increased the time spent eating, ruminating and chewing, probably trying to select the ingredients looking for more fermentable parts. CARVALHO et al. (2006) studied Alpine lactating goats receiving different levels of neutral detergent fiber in the diet, and found a reduction in the time of intake when the diet had greater amount of grain. Therefore, it can be inferred that single pregnancy up to 110 days of gestation did not influence the DMI of ewes, which could facilitate the recovery of animals that are in poor body condition. However, ewes with twin gestation should receive more attention before this stage, especially those with low body condition, and should receive a more caloric diet in order to compensate for the intake reduction. Other hormonal and metabolic factors may also affect this process, but their mechanisms are still controversial and inconclusive.

The ACDDM was affected by the treatments; however, there was no interaction (P> 0.05) between them, indicating the effect of the treatments on this variable. We observed that sheep without nutritional restriction and double pregnancy presented higher digestibility coefficients. This fact is associated with greater amounts of soluble carbohydrates (corn meal), which has high coefficient of digestibility, present in the diet of these animals (Table 1).

The NDF (g/day, % BW and g/kg $BW^{0.75}$) for animals at 110 days of gestation showed a significant difference between treatment and interaction between them ($P < 0.05$). We observed that sheep without nutritional restriction with two fetuses showed lower NDF intake (Table 5). The diet of these animals contained smaller hay content and larger grain content, which consequently decreased the NDF content of the diet, supporting the findings of CARVALHO et al. (2006). Furthermore, lower intake is related to the ingestive capacity of these animals, which possibly suffered the effect of rumen compression by the uterus and appendages. The NDF were 1.88 and 1.01% BW for sheep with one or two fetuses, respectively, receiving unrestricted diet.

Table 5 - Neutral detergent fiber intake and apparent coefficient of digestibility of restricted and unrestricted diets for Santa Ines sheep at 110 days of pregnancy one or two fetuses

	Intake (g/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean
Unrestricted	949.8Aa	550. 9Bb	750.4	1.88Aa	1.01Bb	1.44	50.07Aa	27.35Bb	38.71	58	55	56
Restricted	767.9Ba	816.4Aa	792.2	1.65Aa	1.50Aa	1.58	43.04Aa	40.71Aa	41.87	56	55	55
Mean	858.9	683.6		1.76	1.25		46.55	34.03		57	55	
CV (%)		9.4			9.9			7.7			13.5	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ ($p < 0.05$) by SNK test.

For animals undergoing nutritional restriction, we observed a greater NDFI for animals with two fetuses (816.4 g / day) compared to those with one fetus (767.9 g / day), because the ewes with twin pregnancy have higher nutritional requirements than ewes with single pregnancy, and the animals which suffered nutritional restriction received reduced amount of grains in the diet, forcing them to seek the fibrous part of the diet to meet their needs in energy and protein. Thus, both the number of fetuses and the nutritional management can affect the consumption by animals and, hence, the birth weight of the offspring. Therefore, the separation of animals by the number of fetuses is important to implement the appropriate nutritional management for each category.

The CDNDF was not affected by treatments ($P > 0.05$). This fact indicates that the largest ACDDM observed for the group of animals receiving unrestricted diet was influenced by a denser and more digestible diet offered.

For the sheep at 130 days of pregnancy, we observed that the restriction led to lower dry matter intake by sheep with single pregnancy. DMI was higher for ewes pregnant with two fetuses and under feed restriction than ewes pregnant with two fetuses (1.62 and 1.27 kg/day, respectively). This difference can be attributed to the higher amount of food offered to pregnant ewes with double pregnancy because their requirements are greater (Table 6).

Among animals not subjected to food restriction, there was no difference between ewes pregnant with one and two fetuses, with an average intake of 1.56 kg/day. This result was similar to that observed by SCHEAFFER et al. (2004), who evaluated the consumption of different breeds of sheep, at 130 days of pregnancy, and found a mean intake of 1.46 kg / day.

There were no differences ($P > 0.05$) in the DMI in terms of body and metabolic weight. These results suggest that pregnancy affected the intake, since the ewes were 20 days prior to parturition, when fetal growth occurs exponentially (NRC, 1985).

We observed that the ACDDM was influenced by the number of fetuses. Sheep with two fetuses had higher ACDDM. It is noteworthy that these animals received large amounts of soluble carbohydrates in the diet, which may have increased the ACDDM. According to VALADARES FILHO et al. (1990), nonstructural carbohydrates have total apparent digestibility of over 90% and structural carbohydrates close to 50%.

Table 6 - Dry matter intake and apparent coefficient of digestibility of restricted and unrestricted diets for Santa Ines sheep at 130 days of pregnancy with one or two fetuses

	Intake (kg/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean
						Matéria Seca						
Unrestricted	1.54Aa	1.59Aa	1.56	2.89	2.55	2.72	77,9	71,6	74,7	59	62	60
Restricted	1.27Bb	1.62Aa	1.44	2.65	2.7	2.68	69,6	75,2	72,4	54	61	58
Mean	1.40	1.60		2.77	2.63		73,7	73,4		57b	61a	
CV (%)		8.3			14			11.5			6.1	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ ($p < 0.05$) by SNK test.

MACEDO JÚNIOR et al. (2006) worked with different proportions of forage NDF (NDFf), and observed an increase in DM digestibility in non-pregnant ewes as the amount of NDFf in the diets increased. Another factor that may be associated with greater ACDDM is that ewes with twin pregnancy increase the ability to metabolize ingested nutrients to meet their requirements, besides being in preparation to subsequent lactation, especially in the case of twin pregnancy, when milk yield is higher (NRC, 1985; FRANÇA et al., 2009).

The nutritional management to which the ewes were subjected at 130 days of pregnancy influenced NDFI. The animals undergoing nutritional restriction had higher consumption, probably because the composition of the diets had greater quantity of hay, raising the amount of NDF consumed. SOUSA JUNIOR et al. (2007) studied sheep receiving babassu meal in the diet, and found an increase in NDF intake due to the increase of this ingredient in the experimental diets. Another factor that probably contributed to the result was that this group was fed diet with lower energy and crude protein content, forcing the animals to select, in the food offered, the necessary nutrients to meet their requirements, raising therefore the consumption of the fibrous part of the diet (Table 7).

Table 7 - Neutral detergent fiber intake and apparent coefficient of digestibility of restricted and unrestricted diets for Santa Ines sheep at 130 days of pregnancy with one or two fetuses

	Intake (g/day)			Intake (% body weight)			Intake (g/kg body weight$^{0.75}$)			Digestibility coefficient (%)		
	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean	1fetus	2fetuses	Mean
Unrestricted	878.8	829.9	854.3B	1.65	1.33	1.49B	44.51	37.38	40.95B	50	50	50B
Restricted	895.0	987.5	941.2A	1.87	1.66	1.76A	49.20	45.97	47.59A	52	55	53A
Mean	886.9	908.7		1.76a	1.49b		46.86a	41.68b		51	53	
CV (%)		10.0			15.9			13.5			7.2	

Lowercase letters in the same line and variable and capital letters in the same column and variable differ ($p < 0.05$) by SNK test.

By analyzing the coefficients of digestibility of NDF, we observed that ewes receiving restricted diet had higher value than those fed unrestricted diet (0.53 and 50%, respectively).The digestibility of a diet is related to its intake, as well as to its chemical composition, the forage:concentrate ratio, the number of meals, the physiological state, among others. Thus, we could infer that the higher fiber intake resulted in lower passage rate and consequent increase in digestibility. Another factor that may have influenced this response is the presence of soluble carbohydrates in the diet for sheep which did not undergo nutritional restriction. The interference of non-structural carbohydrates (NSC) in fiber digestion has been observed frequently. The main effects are pH reduction and negative effect on fiber digestion, which may be related to ruminal microbial imbalance, causing an overlay of amylolytic bacteria on fibrolytic bacteria or even the inhibition of enzymes that degrade fiber by the NSC and by the products of their digestion (HOOVER, 1986;

TAMMINGA et al., 1990; ALVES et al., 2003; MACEDO JUNIOR et al., 2010).

The values of ruminal passage rate of digesta (k), mean retention time in the rumen-reticulum (MRTR), and mean retention time in the gastrointestinal tract (MRTG) in sheep at 90 and 130 days of pregnancy are presented in Table 8. Ewes with double pregnancy had greater ruminal passage rates compared to those with single pregnancy in both periods. We verified that during the last third of gestation, passage rate through the rumen was higher compared to the middle third, regardless of the nutritional management employed.

Table 8 - Ruminal passage rates of solids of Santa Ines ewes at different stages of pregnancy with one or two fetuses, submitted or not to dietary restriction, at 90 (middle third) and 130 days (final third) of pregnancy

Thirds of pregnancy		Unrestricted diet				Restricted diet			
		k (%/h)	MRTR	MRTG	R^2 (%)	k (%/h)	MRTR	MRTG	R^2 (%)
Middle	1 fetus	2.64	75.76	80.56	36.3	2.45	81.63	85.56	82.2
	2 fetuses	3.12	64.10	67.60	73.5	2.84	70.42	73.49	84.4
Final	1 fetus	3.20	62.50	65.48	87.1	3.29	60.79	63.42	87.7
	2 fetuses	3.74	53.48	56.51	90	3.48	57.47	60.24	84.9

k = ruminal passage rate; MRTR = mean retention time in the rumen reticulum; MRTG = mean retention time in the gastrointestinal tract; R^2 = coefficient of determination.

We observed that ewes with double pregnancy had higher dry matter intake during pregnancy because of increased demand for nutrients. However, we also verified that these animals showed greater passage of food through the rumen, which contributes to increased DMI. Another factor that may have contributed is the greater presence of grains in the diet of ewes pregnant with two fetuses.

Nutritional restriction reduced the DMI of the animals. We verified higher mean retention time of digesta in the rumen-reticulum (MRTR), probably due to a greater supply of hay in their diet. The MRTR time and in total digestive tract was always lower for ewes with two fetuses, regardless of gestational stage and type of nutritional management. We observed that the animals under dietary restriction in the middle third of gestation of one fetus had higher retention time of digesta in the rumen-reticulum and throughout the digestive tract. These animals received a large amount of hay in their diet, which may have contributed to greater retention of digesta in the rumen. However, in late pregnancy, these animals showed lower retention time of digesta, indicating an adaptive mechanism to try to increase the dry matter intake to meet their nutritional requirements because they were fed a diet with 15% less energy and CP. This adaptive mechanism presented by animals has not been well explained by the literature. Thus, it appears that the diet for pregnant ewes should be prepared in order to maximize their use capacity by the animals.

URESTE (2001) studied Manchega wool sheep and observed 15% increase in retention time of digesta with the advancement of gestation, eight to two weeks before parturition, similar results to those found in this study, in which we verified increase of 17.5% from 90 to 130 days of gestation of ewes with one fetus and unrestricted diet.

Ewes with double pregnancy and fed unrestricted diet had a lower retention time of digesta in the middle third and final pregnancy, possibly due to a large amount of soluble carbohydrates present in their diets. However, when we compared the pregnancy stages, we found lower time in the final third, indicating that these animals were using physiological resources to increase the dry matter intake, since in this stage of pregnancy, physical and physiological factors cause a decrease in dry matter intake.

The coefficients of determination (R^2) were high, indicating that the model used was adequate to the phenomenon, with the exception of animals with one fetus and unrestricted diet in early gestation.

CONCLUSION

Ewes carrying two fetuses ate more and had better digestibility coefficient of dry matter. In the middle term of pregnancy, the dry matter intake is related to the body weight of the ewe; however, in the final third, pregnancy interferes directly on consumption, being reduced mainly in animals carrying two fetuses.

The passage rate of solids increases with the advancing of gestation, a fact that should be explored by nutritionists in balancing rations, especially during late gestation.

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Protocolado em: 04 jun. 2012. Aceito em: 26 out. 2011