

DIET FOR COMPENSATORY GAIN OF SHEEP IN FEEDLOT: BIOMETRY AND MORPHOMETRIC ASPECTS OF THE TESTICLES



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ABSTRACT

The objective of this study was to determine the effect of the diet for compensatory gain on biometry, body condition, scrotal circumference, absolute and relative testicular weight and

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morphometry of seminiferous tubules of lambs finished in feedlot. For this purpose, 40 Santa Inês lambs, non-castrated males, were housed in individual stalls. The feedlot was divided into two distinct periods of 42 days: a feeding restriction period, with four treatments (0, 20, 40 and 60% restriction), and a refeeding period, where all animals were refed without restriction. At the end of the confinement period, the biometrics, body condition, and testicular morphology were evaluated. After slaughter, fragments of the testicles were collected to evaluate the morphometry of the seminiferous tubules. Final weight (44.0, 41.6, 39.4, 35.8 kg), zoometric measurements of body length and chest circumference decreased linearly as the previous restriction increased. The body condition score; the height of the previous one; height of the hindquarter; leg length; thigh circumference; scrotal circumference (mean of 28.4 cm); absolute and relative testicular weight; diameter, circumference and area of the lumen; diameter, circumference and area of the seminiferous tubule; The height of the seminiferous epithelium and the percentage of the cross-sections of the seminiferous tubules containing the different cells of the seminiferous epithelium of lambs were not influenced by the feeding regimen for compensatory gain. The feeding regimen for compensatory gain can be used in feedlot sheep without prejudice to their reproductive condition.

Keywords: Zoometric Measurements. Dietary Restriction. Saint Agnes. Seminiferous tubules.

INTRODUCTION

For many years, sheep farming in the Northeast was treated as a marginal and subsistence activity, practiced by producers of low technical-socio-economic level, whose general panorama was of very low productivity rates. However, currently, sheep and goat farming has acquired an unusual importance in livestock farming in the Northeast Region. However, in order for northeastern sheep farming to become a viable and economically sustainable business, it is necessary to implement measures that overcome the problem of seasonality, quantitative and qualitative, in the production of forage that occurs, notably, in the semi-arid region.

According to Neiva et al. (2005), among the existing options to produce sheep during the drought, a time of scarce forage availability, confinement appears as an alternative for production to be constant and continuous throughout the year. However, this finishing system requires a feed of high nutritional value and, therefore, of high cost. Among the technology options that allow greater efficiency and food economy in feedlot, the use of a diet that results in compensatory gain seems to be one of the most viable. According to Ben Salem and Smith (2008), compensatory gain consists of weight gain above normal, observed in animals submitted to refeeding after a period of food restriction.

On the other hand, one of the main factors to be considered in feedlot and compensatory gain is the performance potential of the animals to be confined and compensated, so that they must respond to the expected gains depending on the diet offered. Among all the native sheep breeds, Santa Inês stands out.

Although several effects of the diet for compensatory gain have been found on domestic ruminants, such as changes in feed conversion (Homem Junior et al., 2007), weight gain (Shadnough et al., 2011), scrotal circumference (Kamalzadeh et al., 1998), viscera size (Almeida et al., 2011); studies of compensatory gain with native sheep breeds of the Brazilian Northeast are still very scarce.

The evaluation of biometrics, through measurements obtained from the live animal, such as body weight, body condition, zoometry (linear and circular), is seen as a great tool to estimate the proportion of meat in the carcass, mainly because it is easy and quick to apply, and can even be carried out on the platform or commercial slaughter line. In a study carried out with sheep in the compensatory gain regime, Kamalzadeh et al. (1998) evaluated the biometric measurements of these animals, and observed variations in the development of the animal's body dimensions during the restriction and refeeding phase,

verifying the influence of the feeding regime on them, in addition to demonstrating the correlation between these measurements.

According to McManus et al. (2010), one of the main factors that can influence testicular morphology is animal nutrition, in addition to observing that heavier animals have larger testicles and, consequently, better values of histological parameters, with seminiferous tubules of larger sizes and a greater number of cells of the spermatogenic lineage. Therefore, the estimation of testicular size is considered an important factor in the reproductive evaluation of sheep, allowing producers to choose the best animals for reproduction (Assis et al., 2008).

In addition, Martins et al. (2008) concluded that testicular biometry is an adequate indicator of spermatogenic capacity; however, the evaluation of the seminiferous epithelium – composed of two basic cell types: the Sertoli cells, whose population is already established before puberty, and the developing germ cells (Hafez and Hafez, 2004) – can indicate, in the set of stages of the seminiferous epithelium cycle, changes in the frequency of germ cells, influenced by environmental and nutritional conditions, because, according to Moghaddam et al. (2012), these are some of the many factors that affect the spermatogenesis process.

In this sense, the objective of this study was to determine the effect of the diet for compensatory gain on biometry, body condition, scrotal circumference, absolute and relative testicular weight and morphometry of the seminiferous tubules of Santa Inês lambs finished in feedlot.

METHODOLOGY

To carry out the experiment, 40 Santa Inês sheep, whole males, weaned, with mean \pm standard deviation of 17 ± 1.7 kg of body weight (BW) and 100 days of age, were used. At the beginning of the experiment, the animals were identified, treated against ecto and endoparasites, and vaccinated against clostridiosis. They were then housed in individual stalls with dimensions of 1.0 x 1.2 m, kept in a shed with cement floors and covered with clay tiles, equipped with feeders and drinkers.

The animals were fed twice a day, at 7 a.m. and 3 p.m. The experimental diet (Table 1), in the form of a complete diet, was formulated based on the requirements of these animals for a gain of 250 g per day, according to the recommendations of the NRC (1985).

Table 1. Proportion of ingredients and bromatological composition of the experimental diet

INGREDIENTS	PROPORTION (%)
Feno de Tifton	30,0
Ground corn	47,0
Farelo de soja	16,5
Wheat bran	4,0
Limestone	1,5
Mineral salt	1,0
BROMATOLOGICAL COMPOSITION	
Dry matter	90,07
Brute protein ¹	16,25
Ethereal Extract ¹	3,17
Metabolizable energy (Mcal/kg DM) ²	2,82
Neutral detergent fibre ¹	63,84
Mineral matter ¹	6,14
<small>¹% in relation to dry matter; ²ME = DE x 0.82, where ME is the metabolizable energy, DE is the digestible energy of the diet (3.44 Mcal/kg of DM, Borburema, 2010) and 0.82 is the metabolizability of the diet (NRC, 2007).</small>	

The experimental period lasted 98 days, between January and April, including 14 initial days of adaptation of the animals to the facilities, management and diet. The remainder is divided into two distinct periods of 42 days each: a period of food restriction (1st to 42nd day) and a period of refeeding (43rd to 84th day). In the period of food restriction, the animals were divided into four treatments, with 0, 20, 40 and 60% restriction, where those in the control treatment, with 0% restriction, received food ad libitum, with daily readjustment that allowed a surplus of 10%, thus ensuring voluntary consumption, and the other treatments followed a diet restriction regime, 20, 40 and 60% in relation to the control treatment. During the refeeding period, all animals, from all treatments, were refed without restriction, that is, at will.

At the end of the confinement period, the animals were weighed to obtain the final weight and, soon after, they were subjected to fasting of 16 hours of water diet and 24 hours of solid diet, followed by the stages of evaluation of zoometry (biometry), body condition, measurement of scrotal circumference and, finally, slaughtered according to techniques and standards recommended by RIISPOA (Brazil, 1997).

The animals' biometry was performed using the following zoometric measurements: body length, anterior height, posterior height, thoracic circumference, leg length and thigh circumference, measured by stick and tape measure (Cezar and Sousa, 2010).

The body condition of the animals was evaluated through visual examination and external palpation of the lumbar region, to estimate the amount of muscle and adipose tissue deposited on the animal skeleton, and subsequent determination of scores from 1 to

5 to quantify this condition, which were considered higher the greater the deposit of muscle and fat (Cezar and Sousa, 2007).

Even before slaughter, the scrotal circumference (perimeter) was measured at the point of greatest diameter (of the pair), by means of a tape measure, with the testicles loose in the scrotum. Immediately after lamb slaughter, the testicles were removed and weighed to determine absolute (g) and relative (g/100g BW) testicular weight. Fragments were then collected and immediately fixed in 10% buffered formaldehyde for 24 hours, in order to avoid postmortem changes in the biological material. After fixation, the specimens were washed under running water and kept in 70% alcohol until the moment of histological processing for paraffin embedding. 3 mm thick tissue fragments were dehydrated in increasing solutions of ethyl alcohol (70 to 100%), diaphanized in xylol and then included in histological paraffin at 58-60°C. The paraffin blocks were taken to the rotating microtome to obtain histological sections with 5 µm thickness. Then, the sections were submitted to the histochemical technique of Periodic Acid-Reactive Schiff (PAS) and the slides were mounted with coverslips on entellan, according to Samuelson (2007).

To measure the tubules and identify the presence of seminiferous epithelium cells, 100 seminiferous tubules per animal were evaluated. Measurements were performed in micrometers (µm) and included the following dimensions: diameter, circumference, and area of the tubule (µm²) and lumen and height of the seminiferous epithelium. Sertoli cells, spermatogonia A and B, spermatocytes, and young and late spermatids were identified. The sections were fully screened and the tubular sections were analyzed randomly and in a sequenced manner to avoid the reexamination of the same area of the section. The image capture, the measurement of the seminiferous tubules and the identification of the cells were done using the computational software Q-Capture and Image-Pro Express 6.0 (Olympus), coupled to a benchtop microscope, Olympus BX41, using 20x and 40x objectives, for measurement and identification, respectively.

The experimental design was completely randomized, with four treatments (considering the diets of the restriction period – 0, 20, 40 and 60%) and ten replications. Statistical analyses were performed through analysis of variance, regression, correlation and mean test (Tukey) for the presence of seminiferous epithelium cells, according to the PROC GLM, PROC REG, PROC CORR and PROC ANOVA procedures of SAS (2003).

RESULTS

The body condition score and zoometric measurements of anterior height, posterior height, leg length and thigh circumference were not influenced by the diet (Table 2).

Table 2. Means and coefficients of variation of final weight, body condition score and zoometric measurements of Santa Inês lambs, submitted to the feeding regimen for compensatory gain, finished in feedlot

Variables	Treatments				CV
	0%	20%	40%	60%	
Final weight (kg)	44,0	41,6	39,4	35,8	10,1 ¹
Body condition score	2,9	2,8	2,7	2,7	13,4
Body Length (cm)	62,4	61,1	59,9	59,4	4,5 ²
Height of anterior (cm)	64,8	64,0	65,8	64,1	3,7
Height of the back (cm)	65,7	64,1	65,4	64,2	2,9
Perímetro torácico (cm)	81,9	80,3	78,5	76,4	4,0 ³
Leg length (cm)	56,3	56,0	56,9	55,5	4,3
Thigh Circumference (cm)	43,2	43,4	43,9	42,0	7,3

*Averages with linear effect (P<0.05); 1y=44.252-0.1344x (r2=0.37); 2y=62.23-0.051x (r2=0.16); 3y=81.74419-0.0843x (r2=0.26)

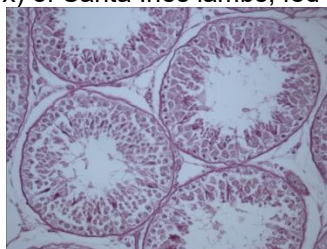
The final weight, zoometric measurements of body length and thoracic circumference decreased linearly as the previous restriction increased, thus, it is understood that the refeeding period was not sufficient to compensate for the loss that occurred during the previous restriction phase, that is, there was no compensatory gain for these variables.

The variables, scrotal circumference, absolute and relative testicular weight, lumen diameter and circumference, seminiferous tubule diameter and circumference, lumen and seminiferous tubule area, and seminiferous epithelium height (Fig. 1) were not influenced by the diet for compensatory gain (Table 3).

Table 3. Means and coefficients of variation of scrotal circumference, absolute and relative testicular weight and morphometry of seminiferous tubules of Santa Inês lambs, submitted to the diet for compensatory gain, finished in feedlot

Variables	Treatments				CV
	0%	20%	40%	60%	
Scrotal circumference (cm)	28,1	29,0	27,5	28,9	12,2
Absolute testicular weight (g)	391,7	385,0	375,8	361,7	25,7
Relative testicular weight (g/100g)	0,9	0,9	1,0	1,0	23,5
Tubular diameter (µm)	308,5	314,4	309,42	311,4	6,3
Tubular circumference (µm)	1 072,5	1 070,8	1 060,2	1 107,5	12,2
Tubular Area (µm ²)	75 699,0	79 077,7	75 449,4	75421,3	13,2
Lumen Diameter (µm)	137,1	140,7	139,2	131,7	8,4
Lumen circumference (µm)	475,6	496,1	506,0	481,9	13,8
Lumen Area (µm ²)	15 580,1	16 612,1	16 062,6	14 414,9	15,4
Seminiferous epithelium height (µm)	87,5	85,6	83,2	90,5	8,7

Figure 1. Seminiferous tubules (SBP, 20x) of Santa Inês lambs, fed for compensatory gain, finished in feedlot



The diet for compensatory gain did not affect ($P>0.05$) the percentage of the cross-sections of the seminiferous tubules that contain the different cells of the seminiferous epithelium of lambs (Table 4).

Table 4. Means and coefficients of variation of the percentage of the cross-sections of seminiferous tubules containing specific types of germ cells of Santa Inês lambs, submitted to the feeding regimen for compensatory gain, finished in feedlot

Variables	Treatments				CV
	0%	20%	40%	60%	
Sertoli Cells	100,0	100,0	100,0	100,0	0,0
Spermatogonia A	51,5	49,5	44,3	46,2	15,2
Spermatogonia B	58,2	65,0	61,8	63,0	11,5
Spermatocytes	59,3	55,5	62,3	65,3	16,6
Young spermatids	86,8	84,8	85,0	84,8	8,7
Late spermatis	65,8	72,7	73,7	68,1	7,9

Germ cells, surrounded by Sertoli cells during development, undergo a continuous series of cell divisions and modifications (spermatogenesis), which begins in the periphery and progresses towards the tubular lumen, evolving from spermatogonia, through spermatocytes (spermatocytogenesis), spermatids, to spermatozoa (spermiogenesis), being present in different forms and frequencies in the various stages of the seminiferous epithelium cycle (Hafez and Hafez, 2004).

Body weight showed a significant positive correlation with zoometric measurements ($P<0.01$), except for hind height, and with testicular weight ($P<0.01$), i.e., heavier animals had higher zoometric measurements and higher testicular weight (Table 5). However, the body condition score had a significant positive correlation only with the thoracic perimeter ($P<0.01$).

Table 5. Correlation coefficients between biometric parameters of Santa Inês lambs, submitted to the feeding regimen for compensatory gain, finished in feedlot

Var.	CC	CO	AA	AP	PT	CP	CX	THAT	PTE
PC	0,32	0,48**	0,49**	0,28	0,82**	0,50**	0,45**	0,30	0,46**
CC	-	0,09	-0,11	-0,07	0,46**	-0,14	0,30	0,16	0,22

Var = variables; WC = body weight; WC = body condition score; CO = body length; AA = height of the anterior; AP = posterior height; PT = thoracic circumference; NC = leg length; CX = thigh circumference; EC = scrotal circumference; PTE = weight of the testicles. $P < 0.01^{**}$.

The correlation between body weight and zoometric measurements makes body weight a better predictor of carcass tissue composition, as this alone is not a good parameter for this (Cezar and Sousa, 2007).

Scrotal circumference showed a significant positive correlation only with testicular weight ($P < 0.01$), thus suggesting that the greater the testicle circumference, the greater its weight (Table 6). Assis et al. (2008) reported a high correlation between these two parameters and considered that the prediction of testicular weight through scrotal circumference is very reliable and useful for the evaluation of animals regarding their reproductive capacity, as it has presented a higher correlation coefficient, it is more efficient than the prediction through body weight.

Table 6. Correlation coefficients between the morphology of the testicles and seminiferous tubules of Santa Inês lambs, submitted to the diet for compensatory gain, finished in feedlot

Var	PTE	DT	CT	AT	DL	CL	AL	EP
CE	0,78**	0,21	0,28	0,18	-0,11	0,26	-0,06	0,16
PTE		0,28	0,34*	0,27	-0,10	0,31	-0,07	0,25
DT			0,80**	0,98**	0,51**	0,73**	0,51**	0,70**
CT				0,76**	0,35	0,86**	0,33	0,65**
AT					0,53**	0,71**	0,54**	0,67**
DL						0,71**	0,97**	-0,18
CL							0,72**	0,24
AL								-0,20

Var = variables; EC = scrotal circumference; PTE = testicle weight; TD = tubule diameter; CT = tubule circumference; AT = tubule area; DL = lumen diameter; CL = lumen circumference; AL = lumen area; PE = height of the epithelium. $P < 0.01^{**}$; $P < 0.05^*$.

DISCUSSION

The reduction in final weight in the different treatments is certainly due to the previous increasing dietary restriction, with a consequent reduction in consumption and daily weight gain. This result differs from that obtained by Almeida et al. (2011), who, working with Santa Inês sheep in a compensatory gain regime, observed growth compensation after feeding restriction, and concluded that this compensation may vary according to the degree of maturity in which the animals are, a fact that may explain the divergence between the results obtained. There are also other factors that can influence the rate of compensatory gain, including breed, period and severity of the restriction, duration and quality of refeeding (Shadnoush et al., 2011).

It is considered, with the results related to the zoometric measurements, that the proportion of the animal's trunk decreased, while the body extremities were maintained, which could result in animals with lower carcass yields, since the trunk is a body region of the sheep that originates more integral parts of the carcass than its body extremities. This fact has already been reported by Almeida (2010), where the carcass yield decreased as the level of prior restriction increased. In addition, when comparing the measurements of body length and thoracic circumference with the heights of the posterior and anterior, we can infer that the previous restriction resulted in more wading animals, thus resulting in estimates of lower yields of the edible portion of the carcass, given the lower compactness of the leg. However, even considering the animals with the highest previous restriction (60%), these measurements remained close to those obtained by Sousa et al. (2009), for Santa Inês lambs, finished in feedlot, slaughtered in the same weight range.

The feeding restriction to which the lambs were subjected in the first stage of confinement was not sufficient to affect the morphometric measurements of the testicles and seminiferous tubules of these animals ($P>0.05$). An inverse result was observed by Carrijo Junior et al. (2008), where the diet with low levels of protein decreased the reproductive measurements of Santa Inês lambs, and by McManus et al. (2010), who noticed that Santa Inês lambs had the development of the gonads influenced by variations in food availability. However, the lambs were already on average 15 weeks old, probably with testicle sizes already stabilized, because according to Hafez and Hafez (2004), the size of the sheep's testicles increases when they reach eight to 10 weeks of age and live weight of 16 to 20 kg, coinciding with the appearance of primary spermatocytes and elongation of the seminiferous tubules.

Puberty in sheep is associated with marked increases in testosterone, spermatogenesis, and mounting behavior, and usually occurs in lambs four to six months of age, with weights ranging from 40 to 60% of adult weight, resulting in copulation with viable sperm ejaculation (Hafez and Hafez, 2004).

Sertoli cells are located next to the basement membrane of the seminiferous tubules, their cytoplasm surrounds the germ cells, and extends to the tubular lumen, with functions such as formation of the blood-testicular barrier and the structural and nutritional support of the germ cells, and are present in all stages of the seminiferous epithelium (Costa and Paula, 2003), as shown in Table 4.

Table 4 shows that the percentage of the cross-sections of the seminiferous tubules containing the different germ cells was equivalent at the different levels of previous restriction, i.e., the diet did not cause histological alterations that could affect spermatogenesis; This result differs from that obtained by McManus et al. (2010), where variations in food availability were directly reflected in the histological structure of the testicles of Santa Inês lambs. However, the results obtained in this experiment indicate that the compensatory gain feeding regimen can be used with the objective of reducing the production costs of sheep in feedlot, thus minimizing the impact of drought on the performance of these animals, without prejudice to their reproductive condition.

A positive correlation between body weight and testicular weight was also observed by Assis et al. (2008) and Jafariahangari et al. (2012), both evaluating reproductive traits of sheep. Silvestre et al. (2012) considered that the high correlation between these parameters demonstrates the influence of the qualitative and quantitative contribution of food on the development of the gonads of male goats. This correlation denotes that heavier animals have larger testicles and, consequently, body weight can be considered as an efficient indicator of this characteristic (McManus et al., 2010), and considered as important for the evaluation of rams for reproduction as the analysis of sperm production (Allaoui et al., 2014).

Among the morphological measurements of the seminiferous tubules, there was a significant positive correlation between the tubule and lumen measurements, and between the tubule and epithelium height ($P < 0.01$), results similar to those observed by McManus et al. (2010) and Carrijo Junior et al. (2008), in studies with Santa Inês sheep.

Martins et al. (2008), with results different from those of the present study, noted high correlations between testicular biometry and histological characteristics of the tubule and seminiferous epithelium, associating larger gonads with better spermatogenesis efficiency, and concluded that testicular biometry is an adequate indicator of the other structures of the reproductive tract and spermatogenic capacity of woolly lambs.

CONCLUSION

The feeding regimen for compensatory gain reduced body length and chest circumference, but did not affect the body condition of the lambs; nor did it alter the morphometric measurements of the testes, the morphometry of the seminiferous tubules and the percentage of the cross-sections of the seminiferous tubules that contain the

different cells of the seminiferous epithelium of Santa Inês lambs, finished in feedlot. Body weight showed a positive correlation with testicular weight, which in turn had a high positive correlation with scrotal circumference. The feeding regimen for compensatory gain can be used in feedlot sheep without prejudice to their reproductive condition.

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