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REGULAR ARTICLES

Productive performance and reproductive characteristics of Morada Nova male lambs fed with high-energy diet

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Abstract

Morada Nova breed sheep are without wool, tropicalized, small-sized animals, known for their high-quality meat and skin. Their body development naturally depends on the genetic potential and adequate nutritional support, which suggests that the offer of high-energy density diets positively influences their productive indicators. Thus, the present study investigated the effect of a high-energy diet for the Morada Nova lambs on body development and testicular function, considering their histomorphometric characteristics and seminal quality. Forty-two males (19.2 weeks, 20.7 ± 3.5 kg) were equally divided into two groups and fed with 2.05 Mcal (G7, $n = 21$) or 2.37 Mcal (G24, $n = 21$) of metabolizable energy/day, equivalent to 7% and 24% above the minimum for growing lambs. The animals were confined for 23 weeks (W0 to W23). Weight and body score differed significantly from the W1 ($P < 0.05$). From the W5, thoracic perimeter, body length, wither height, and rump attributes were higher in G24 ($P < 0.05$). The scrotal circumference and testicular volume were higher in G24 from the W3 ($P < 0.05$). Although testosterone levels were not affected ($P = 0.05$), the highest energy intake increased the diameter of the seminiferous tubules and the development of the epididymal epithelium ($P < 0.05$). This positively influenced the seminal quality and reduced the minor defects (21.87% vs. 17.13%) and the total spermatic defects (26.34% vs. 21.78%, $P < 0.05$). Thus, it is possible to employ higher levels of dietary energy for Morada Nova young males to express higher productive efficiency and earlier reproductive attributes of interest.

Keywords *Ovis aries* · Hair sheep · Performance · Morphometry · Sperm

Introduction

Domesticated sheep have developed slowly over thousands of years through natural selection and more rapidly after human intervention. During the development of a sheep breed, many characteristics result from different selection pressures, becoming permanent attributes (Arandas et al. 2017). Among the traits of zootechnical interest, productivity and reproductive precocity are highly relevant, as better performance ensures that the animal reaches faster slaughter weight or presents development characteristics to go early into reproductive stage.

In tropical regions, sheep herds are largely represented by wool-less animals, among which the Morada Nova breed presents one of the main naturalized genetic resources, originating in the northeast of Brazil (Paiva et al. 2005; Gonzaga Neto et al. 2006). Its most positive aspect is adaptation to the

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climatic adversities imposed by the semi-arid regions and other localities with higher temperatures and mountainous conditions (Facó et al. 2008). They are small-framed animals, exploited for meat production and high-quality skin (Jacinto et al. 2004). However, it is common for these animals to be raised in production systems with low technological level, which can underestimate their productivity indicators (Freitas et al. 2005). Due to its importance as a tropicalized genotype, the breed has been attracting attention and more scientific studies on Morada Nova animals have been published. The emerging scientific evidences can value this breed as an important and resilient genetic resource for breeding programs. This would also allow the preservation and expansion of the current Morada Nova herds (Costa et al. 2013; Ribeiro and González-García, 2016).

The animal performance depends on the interaction between genotype, nutrition, and physiological conditions (Santos et al. 2006). Thus, it is necessary to adapt the nutritional management to each exploration situation of the sheep-raising (Piola Junior et al. 2009). Among the effects of nutrition on sheep production, energy supplementation influences weight gain, which is essential for the functioning of vital organs, cell activity and renewal, as well as in the nutrient utilization (Mahgoub et al. 2000) and reproductive processes (Pires 2011). Since body development depends on the genetic potential of the animals and fundamentally on an adequate feed supply, in terms of quantity and quality (Facó et al. 2008), it can be hypothesized that the provision of higher-energy-density diets and above the minimum levels for Morada Nova lambs positively influences their body development and reproductive traits.

Therefore, the objective of this work was to study the influence of dietary energy content on body development and gonadal function, as well as to evaluate the seminal quality and the histomorphometric characteristics of the testicles and epididymis of Morada Nova lambs.

Material and methods

Location and trial period

The experiment was conducted at Embrapa Southeast Livestock, São Carlos, SP, Brazil (21° 58′ 30″ South, 47° 50′ 58″ West, 911 m altitude). The local climatic subtype is tropical altitude (Cwa), characterized by dry winters and hot rainy summers. The experimental period covered the months from August 2016 to January 2017, totaling 23 weeks.

Bioethics

The experimental procedures were previously approved by the Commission of Ethics in the Use of Experimental Animals of Embrapa Southeast Livestock (Declaration CEUA-CPPSE 06_2013), considering legal and ethical aspects of the interventions carried out. The results were reported according to The Animals in Research: Reporting In Vivo Experiments Guidelines—ARRIVE (Kilkenny et al. 2010). The experiment was carried out in accordance with the Brazilian genetic patrimony access law (SISGEN Registration A0C33B1).

Experimental animals and facilities

A total of 42 Morada Nova male lambs, not castrated and weaned (19.2 weeks, 20.7 ± 3.5 kg), were used. The animals were selected from a herd of 120 lambs to compose a homogeneous experimental batch. A selection criterion was initially adopted, based on sex, age, live weight, and body condition score, to allow the composition of homogeneous experimental groups. Following this first step, which guaranteed a balanced batch of animals, the animals were divided in two experimental groups each containing 21 contemporary males. The number of animals in each group had been previously determined to enable a suitable statistical analysis. The animals were kept in confinement in a covered shed. The shed had an area of 570 m^2, two distinct collective stalls, waterproof concrete floor, sand beds, collective feed troughs, and automatic drinking fountains.

Treatments and experimental diet

The experimental diet was formulated according to the requirements of the National Research Council (NRC 2007). The animals were distributed in two experimental groups, which differed in terms of the energy offered in the diet. For the estimation of metabolizable energy intake, total digestible nutrient intake (TDN) values were converted to digestible energy and then to metabolizable energy, assuming that 1 kg of TDN equals 4.4 Mcal of digestible energy, and then multiplied by 0.82 to obtain ME consumed, according to Selvaraju et al. (2012). Thus, animals from G7 ($n = 21$) received 2.05 Mcal of metabolizable energy (ME) daily, which is equivalent to 7% of energy above the minimum established for growing lambs. On the other hand, animals from G24 ($n = 21$) received 2.37 Mcal of metabolizable energy daily, consisting of an extra quota of 24% of energy, regarding the minimum established for growing lambs.

Corn silage (*Zea mays*) cultivar LC-34 was the feed used, produced in a trench silo. The concentrate provided consisted of ground corn (85.75%), soybean meal (12.17%), mineral supplement (1.16%), limestone (0.93%), and monensin sodium (0.0005%). The animals were fed twice a day (08:00 and 16:00 h), and the diets were offered in collective troughs, which provided linear 0.28 m/animal access. Water and mineral supplementations were permanently available. A 14-day adaptation period of the animals to the facilities and feeding was adopted, when the feed intake was measured, in order to allow 10% of leftovers. There was a daily monitoring and adjustment of the amount offered.

Samples of the feed offered and leftovers were collected weekly, weighed, identified, and stored at − 15 °C for further analysis. The samples of the feed and of the leftovers were homogenized to provide a composite sample of each month. The samples were pre-dried in a forced circulation oven at 65 °C for 72 h and then processed in a 1-mm mesh knife mill. The chemical composition of the corn silage and concentrate was evaluated in duplicate, considering the levels of dry matter (DM, %), organic matter (OM, %), mineral matter (MM, %), crude protein (CP, %), ether extract (EE, %), neutral detergent fiber (NDF, %), and acid detergent fiber (ADF, %), according to the methods of the National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al. 2012). Leftover samples were evaluated for dry matter content (DM, %). The results of the analysis of the feed provided and the leftovers were presented as the means of the collection months (Table 1). The average dry matter intake per experimental group was calculated by the difference between the weight of the feed offered and the leftovers, which were 0.617 kg/animal/day for G7 and 0.587 kg/animal/day for G24.

Body weight and morphometry

Every 2 weeks, the animals were taken to the management pen and, after a 16-h feed fast, their body weight (BW, kg) was individually checked in a decimal scale. Concomitantly, animals were evaluated for body condition score (BCS, 1–5) through visual examination and palpation of the lumbar region (Cezar and Souza 2007). Then, each animal was measured to determine (a) thoracic perimeter (TP, cm) measured using a flexible measuring tape along the contour of the thorax to tangentiate the olecranon; (b) body length (BL, cm), measured with a hypometer from the tip of the scapula to the ischial tuberosity; (c) withers height (WH, cm) measured with a hypometer—distance from the ground to the dorsal end of the spinous processes; (d) rump height (RH, cm) measured with a hypometer—distance from the ground to the highest end of the rump; (e) rump width (RW, cm) measured with flexible measuring tape—maximum distance between the trochanters of both femurs; and (f) rump length (RL, cm) measured with a flexible measuring tape—distance between the ischial and ileum tuberosities (Oliveira et al. 2007). All the measurement instruments used had a millimeter scale.

Table 1 Chemical composition of corn silage, concentrate, and diets offered for Morada Nova lambs, based on dry matter (%DM)

Nutrients	Corn silage	Concentrate	G7 diet	G24 diet
DM (%)	47.55	79.69	53.98	73.23
MM (%)	3.3	3.7	3.38	3.62
OM (%)	96.7	96.3	96.62	96.38
CP (%)	7.26	14.82	8.76	13.3
NDF (%)	62.68	45.15	59.17	48.65
ADF (%)	57.73	42.43	54.64	45.48
EE (%)	3.22	3.8	3.33	3.68

G7 diet with 7% increase in metabolizable energy, *G24* diet with 24% increase in metabolizable energy (reference value: NRC 2007), *DM* dry matter, *MM* mineral matter, *OM* organic matter, *CP* crude protein, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *EE* ether extract

Scrotal circumference and testicular biometry

The scrotal circumference (SC, cm) evaluation used a flexible measuring tape graduated in millimeters. The testicular biometry included the evaluation of testicular length (TL, cm) and testicular width (TW, cm), measured with a metallic caliper. The testicular volume (TV, cm^3) was calculated by the equation [$TV = (4\pi / 3) \times (TL / 2) \times (TW / 2)^2$] according to Bailey et al. (1998). Right and left testicles were measured and the results of testicular biometry were presented as the means of contralateral testes.

Serum testosterone concentration

Blood samples were collected monthly in the morning (08:00 to 09:00 h), by jugular venipuncture, in vacuum tubes without an anticoagulant. The samples were centrifuged at 3600 rpm for 30 min to separate the serum, fractionated in aliquots, and stored in polypropylene microtubes at − 20 °C for subsequent analysis. Testosterone concentrations (ng/mL) were determined by radioimmunoassay using the ImmuChem Double Antibody Testosterone kit (MP Biomedicals, Inc., Diagnostics Division, USA). The detection limit and intra-experiment coefficient were 0.03 ng/mL and 12.2%, respectively.

Semen quality

For the evaluation of seminal quality, the semen of all animals was collected by electroejaculation at weeks 16, 18, and 23. The semen collection success rate (SR, %) was calculated by the ratio between the number of animals that ejaculated and the total number of animals collected × 100. The volume of the ejaculate (VOL, mL) was immediately evaluated in a graduated conical tube. Seminal samples were kept at controlled temperature at 37 °C during the execution of the immediate analyses, when the gross motility (GM, 0 to 5) under light-field microscopy (E-100, Nikon, Japan) with ×40 magnification, the sperm vigor (VIG, 1 to 5), and progressive motility (PM, %) with ×100 magnification were evaluated (CBRA, 2013). Sperm concentration (CONC, $\times 10^9$ sptz/mL) was evaluated by counting in a Neubauer chamber (1:100 dilution). The analysis of the sperm morphology was performed by the wet mount technique, after fixation of fresh semen aliquot in buffered formalin solution, previously heated at 37 °C. Cells were evaluated under phase contrast microscopy (E-200, Nikon, Japan) with magnification of ×1000. Abnormal cells were classified according to the morphological characteristics, such as minor defects (MiDef, %), major defects (MaDef, %), and total defects (TDef, %) (Blom 1973). The results were summarized as the average of the semen collection weeks.

Histomorphometry of testicles and tail of the epididymis

At the end of the experimental period (week 23), 20 animals were selected at random, 10 from G1 and 10 from G2, to evaluate the histological characteristics of the testis and the tail of the epididymis. The testicles and epididymis were surgically removed and dissected. Subsequently, a 5-cm fragment was collected from the middle region of the testis (Fontoura et al. 2016) and the tail of the right and left epididymis. The fragments were fixed in 10% formalin (Prophet et al. 1992) and submitted to transverse sections of 5 mm thickness and placed on microscope slides. The histological sections were stained by the hematoxylin-eosin technique (Fontoura et al. 2016; Li et al. 2017) and were evaluated under light microscopy with ×100 and ×400 magnification. The images were scanned in a coupled color camera (Leica DME and Leica ICC50HD, Leica Microsystems, Germany) and serially and standardized photodocumented and later analyzed using specific software (Leica LAS EZ Version 3.4, Leica Microsystems, Germany).

For testicular analysis, two sections per animal were used, one section for each contralateral testis. In each sample, 100 seminiferous tubules were evaluated per animal, 50 tubules from the right testicle and 50 tubules from the left testicle. The considered tubules showed completely transverse sections, with the presence of a lumen and a difference of < 20% between the major and minor tubule diameters (Rojas-García et al. 2010). The images of each tubule were photodocumented individually for micromorphometric analysis. For each image, the seminiferous tubule diameter (STD, μm) and the lumen diameter (LD, μm) were measured at two distinct points. The thickness of the seminiferous epithelium (SET, μm) was determined after four measurements in each image.

For analysis of the tail of the epididymis, four sections per animal were used, two sections for each contralateral structure. In each sample, 100 epididymal ducts were evaluated per animal, 50 ducts of the right epididymis and 50 ducts of the left epididymis. Four hundred images were scanned per animal, and the thickness of the epididymal epithelium (EET, μm) was determined in eight different points. Histomorphometric analysis was performed using the ImageJ® imaging software (US National Institutes of Health, NIH, Bethesda, MD, USA). Histomorphometric results from the seminiferous tubules and tail of the epididymis were presented as the means of the contralateral structures.

Statistical analysis

The data from a completely randomized design were submitted to analysis of variance using the SAS software MIXED procedure, version 9.4 (SAS 2012). For the studied variables (body weight, body condition score, body morphometry, testicular biometry, testosterone, and seminal quality), the treatment effect and experimental weeks were considered, with their respective interactions, following a split-plot structure

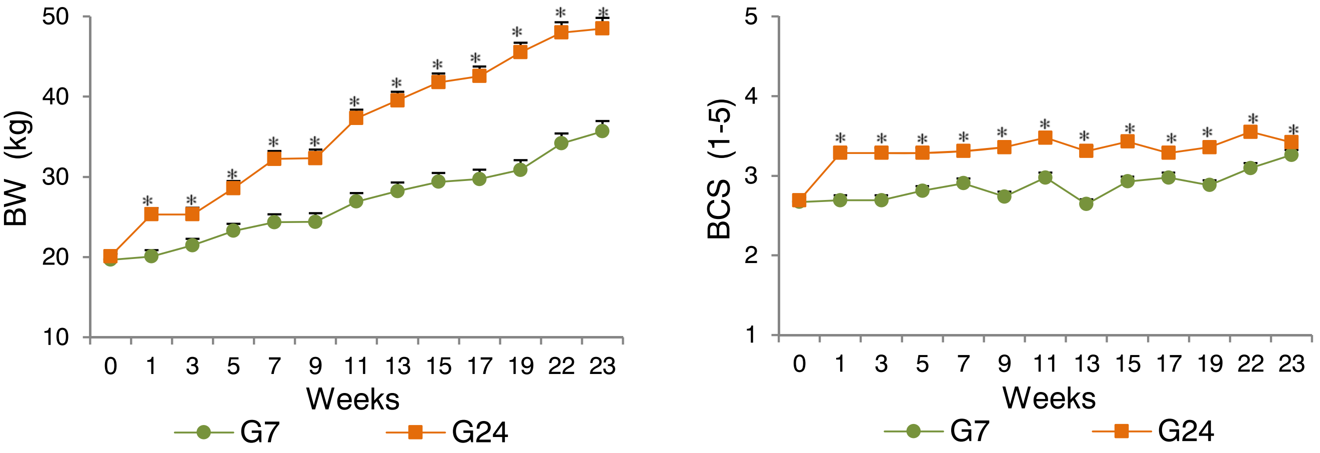

Fig. 1 Mean values (± standard error) of body weight (BW) and body condition score (BCS) of Morada Nova lambs submitted to different energy levels in the diet (G7 and G24 = 7% and 24% increase in metabolizable energy, respectively, according to NRC 2007). Asterisk indicates significant difference between treatments ($P < 0.05$). $n = 21$ animals per group

in time, taking the week as a repeated measure. In choosing the structure of the respective variance and covariance matrices, the criterion of the lowest AIC value was adopted (Akaike 1974). For the analyses, the mathematical model $Y_{ijk} = \mu + T_i + A(T)_{ik} + W_j + T \times W_{ij} + e_{ijk}$ was adopted, where T = treatment, A = animal, and W = week, in which treatment and week were considered fixed effects, while animal was considered random effect. The histomorphometric data were submitted to

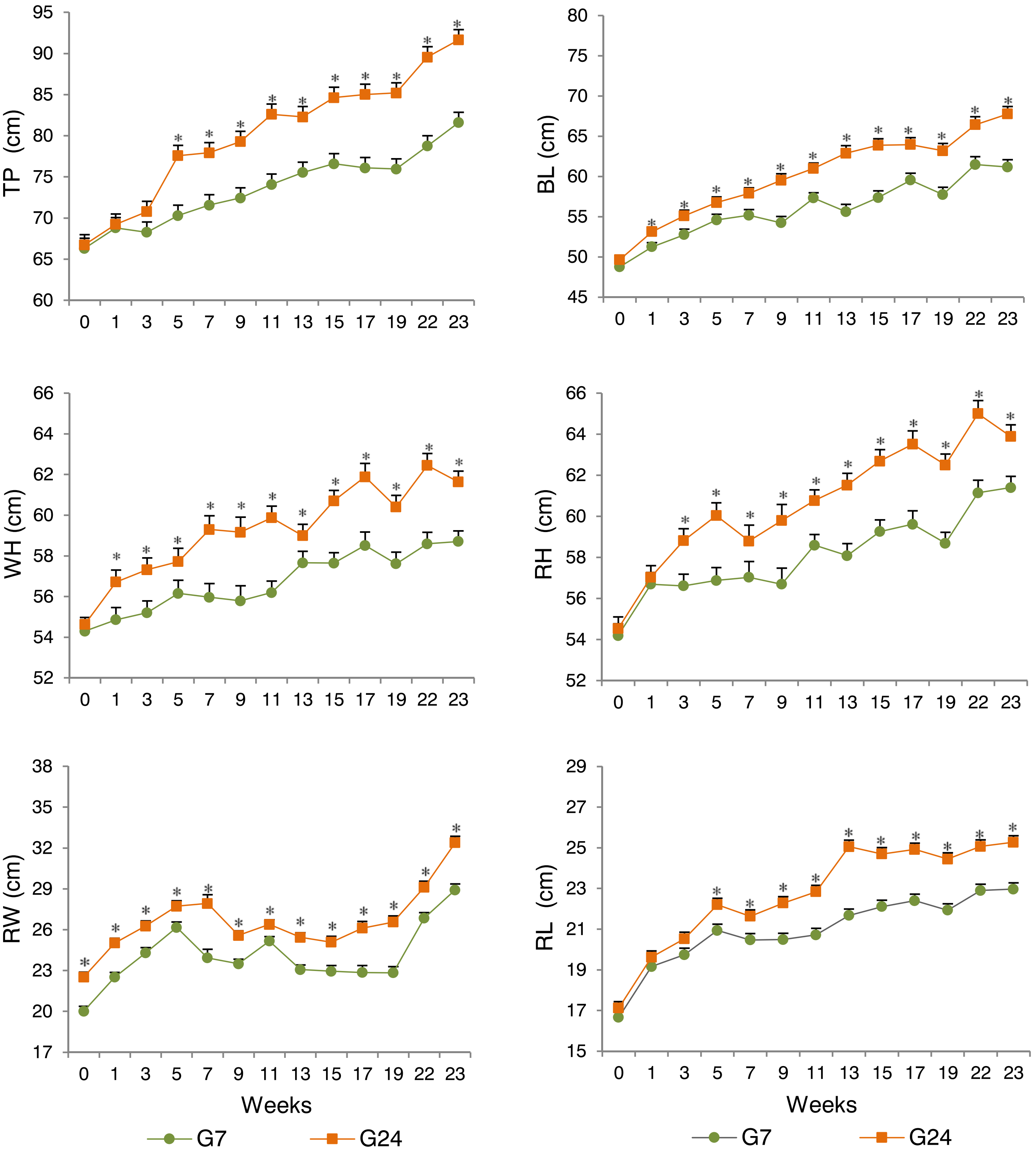

Fig. 2 Mean values (± standard error) of thoracic perimeter (TP), body length (BL), withers height (WH), rump height (RH), rump width (RW), and rump length (RL) of Nova Morada lambs submitted to different energy levels in the diet (G7 and G24 = 7% and 24% increase in metabolizable energy, respectively, according to NRC 2007). Asterisk indicates significant difference between treatments ($P < 0.05$). $n = 21$ animals per group

the GLM procedure considering the treatment effects and the contralateral anatomical structures. For the multiple comparisons between the means, the Tukey test was adopted in the LSMEANS option of the SAS program. The level of significance adopted in all analyses was 5%.

Results

The live weight and body condition score did not differ between treatments at the starting point, demonstrating the homogeneity and efficiency in the distribution of the animals. After the week 1, there was a significant difference between treatments for body weight and body condition score (Fig. 1), with higher values observed for animals in G24.

There was a significant difference between treatments for body length and height at withers after week 1. The thoracic perimeter and rump length differed between treatments from week 5, while the difference for rump height was significant at week 3 for G24 (Fig. 2). The rump width was the single variable, among the 12 morphometric features studied, that did not present a balanced condition between groups at the experimental starting point.

There was a significant difference between treatments for scrotal circumference, testicle length and width, and testicular volume (Fig. 3). The behavior of these variables showed similarity during the experimental period, with a difference between groups from the week 3 and higher values always recorded for high-energy animals.

There was no significant difference between treatments regarding serum testosterone concentration, independently of the week evaluated (Fig. 4).

In relation to the seminal quality evaluation, significant difference was observed for the parameters of minor defects and total spermatic defects between the diets provided (Table 2).

The histomorphometric evaluation showed that the variables of seminiferous tubule diameter and lumen diameter differed between treatments, with higher values for high-energy animals (G24). The thickness of the epididymal epithelium also showed significant difference between treatments (Fig. 5), which was greater for the G24 group.

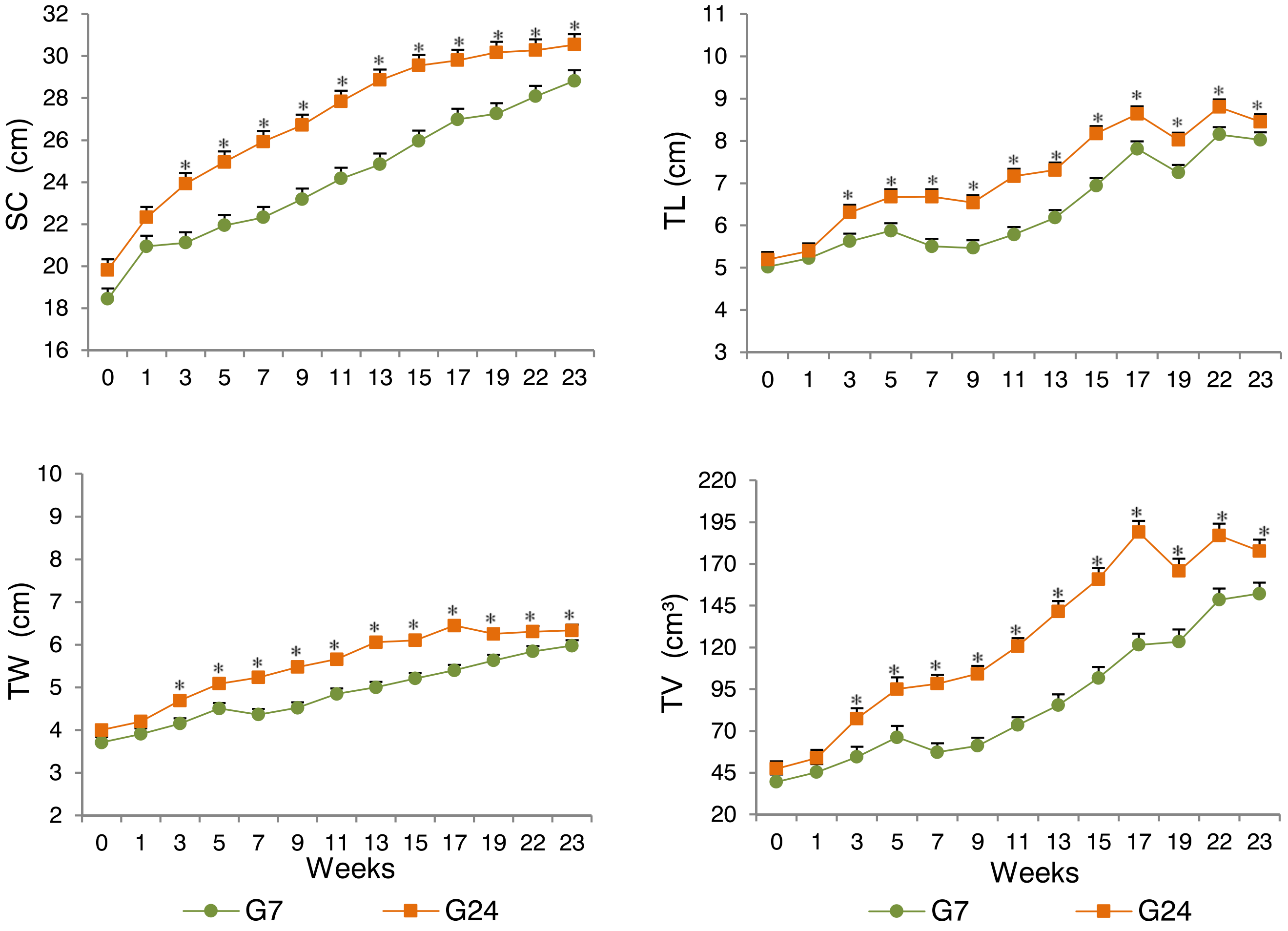

Fig. 3 Mean values (± standard error) of scrotal circumference (SC), testicular length (TL), testicular width (TW), and testicular volume (TV) of Morada Nova lambs submitted to different energy levels (G7 and G24 = 7% and 24% increase in metabolizable energy, respectively, according to NRC, 2007). Asterisk indicates significant difference between treatments ($P < 0.05$). $n = 21$ animals per group

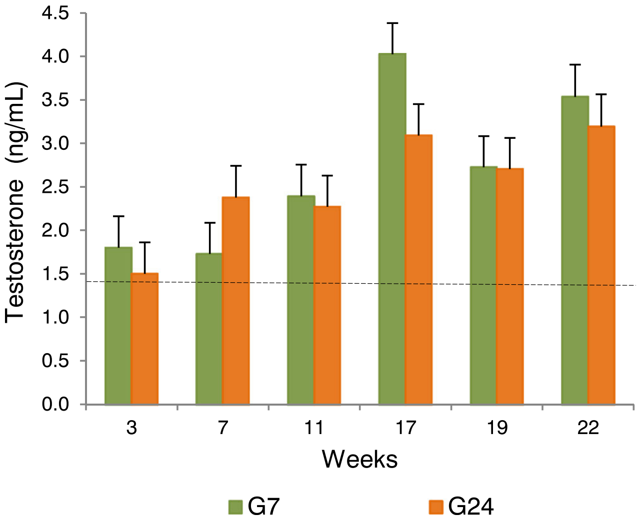

Fig. 4 Means (± standard error) of the testosterone serum concentration of Morada Nova lambs submitted to different energy levels in the diet (G7 and G24 = 7% and 24% increase in metabolizable energy, respectively, according to NRC, 2007). The dashed line demonstrates reference value for rams (Schanbacher et al., 1974). Asterisk indicates significant difference between treatments ($P < 0.05$). $n = 21$ animals per group

Discussion

The successful production of small ruminants in tropical regions depends mostly on the production potential of these animals (Tedeschi et al. 2010). However, their performance is subject to factors such as feeding level, genotype, sex, age, and productive aptitude (NRC 2007). In the present study, animals that received a high-energy diet presented greater weight gain and a more pronounced development of the body score, similarly to that reported in previous studies (Mahgoub et al. 2000; Araújo Filho et al. 2007; Sousa et al. 2012; Bhatt et al. 2013). Due to the fact that it was composed of a higher ratio of concentrate, the diet given to G24 showed a higher amount of non-fibrous carbohydrates, leading to the production of high propionate and low acetate in the rumen during the digestive process (Banskalieva et al. 2005). When absorbed, these volatile fatty acids become the main substrate for the hepatic gluconeogenesis, which results in greater energy availability in the form of glucose. Consequently, this promotes greater daily weight gains (Sousa et al. 2012) and higher rate of growth and fat deposition (Clementino et al. 2007). This positive effect was observed in animals that received high dietary energy, with significant differences in the short term and that intensified throughout the experimental period. It is also possible to consider a complementary effect of higher protein input related to the diet offered to G24 animals.

Table 2 Means (± standard error) of success rates for semen collection and sperm characteristics of Morada Nova lambs ($n = 42$) submitted to different energy levels in the diet

Variable	Treatments		
	G7	G24	*P* value
SR (%)	79.37	88.52	0.07
VOL (mL)	1.23 ± 0.08	1.34 ± 0.08	0.37
GM (0–5)	2.71 ± 0.15	2.78 ± 0.14	0.72
CONC (×10^9 sptz/mL)	1.37 ± 0.12	1.56 ± 0.12	0.27
PM (%)	48.06 ± 3.21	46.63 ± 3.09	0.75
VIG (0–5)	2.54 ± 0.08	2.54 ± 0.07	0.95
MaDef (%)	4.30 ± 0.49	4.73 ± 0.47	0.53
MiDef (%)	21.87 ± 1.49a	17.13 ± 1.43b	0.02
TDef (%)	26.34 ± 1.38a	21.78 ± 1.33b	0.01

Different lowercase letters on the same line indicate significant difference between treatments ($P < 0.05$)

G7 diet with 7% increase in metabolizable energy, *G24* diet with 24% increase in metabolizable energy (reference value: NRC 2007), *SR* success rate, *VOL* sperm volume, *GM* gross motility, *CONC* concentration, *PM* progressive motility, *VIG* spermatic vigor, *MaDef* major defects, *MiDef* minor defects, *TDef* total defects

Understanding and monitoring the phenotypic variables are extremely relevant and provide important information regarding the aspects of adaptation, conformation, and functionality of the animal (Fitzhugh 1978; Sowande and Sobola 2008). The body-morphometric elements studied showed different variations, with a significant difference between treatments, throughout the weeks. At week 0, considered the starting point of the experiment, most of the variables (except for rump width) presented no statistical difference between groups. Throughout the experiment, some variables demonstrated higher responsiveness to the extra quota of energy provided. In fact, body length and withers height were early indicators of morphometric distinction, followed by rump characteristics and body length. Despite certain peculiarities observed in the behavior of the morphometric variables, animals with high-energy intake always presented greater body development, mainly after week 5 of the treatment. This demonstrates that the diet containing highest energy provided the animals greater musculoskeletal growth and that its use allows Morada Nova lambs to reach slaughter weight (35 kg) after 9 weeks of treatment, when they are still young, at 7 months of age, anticipating this indicator by 14 weeks, when compared to G7 animals. This fact is even more relevant when considering reports that indicate some delay in the slaughter age and weight in animals of this breed (Facó et al. 2008; Araújo et al. 2017). The use of this information in selection programs would allow differentiating animals of superior zootechnical performance (Cam et al. 2010), a relevant procedure for the selection of future breeding herds. Measurements such as thoracic perimeter, withers height, rump height, and rump width are important for establishing animal aptitude, since these features relate to the productive capacity and mainly to the production of noble meat cuts (Araújo Filho et al. 2007; Pinheiro

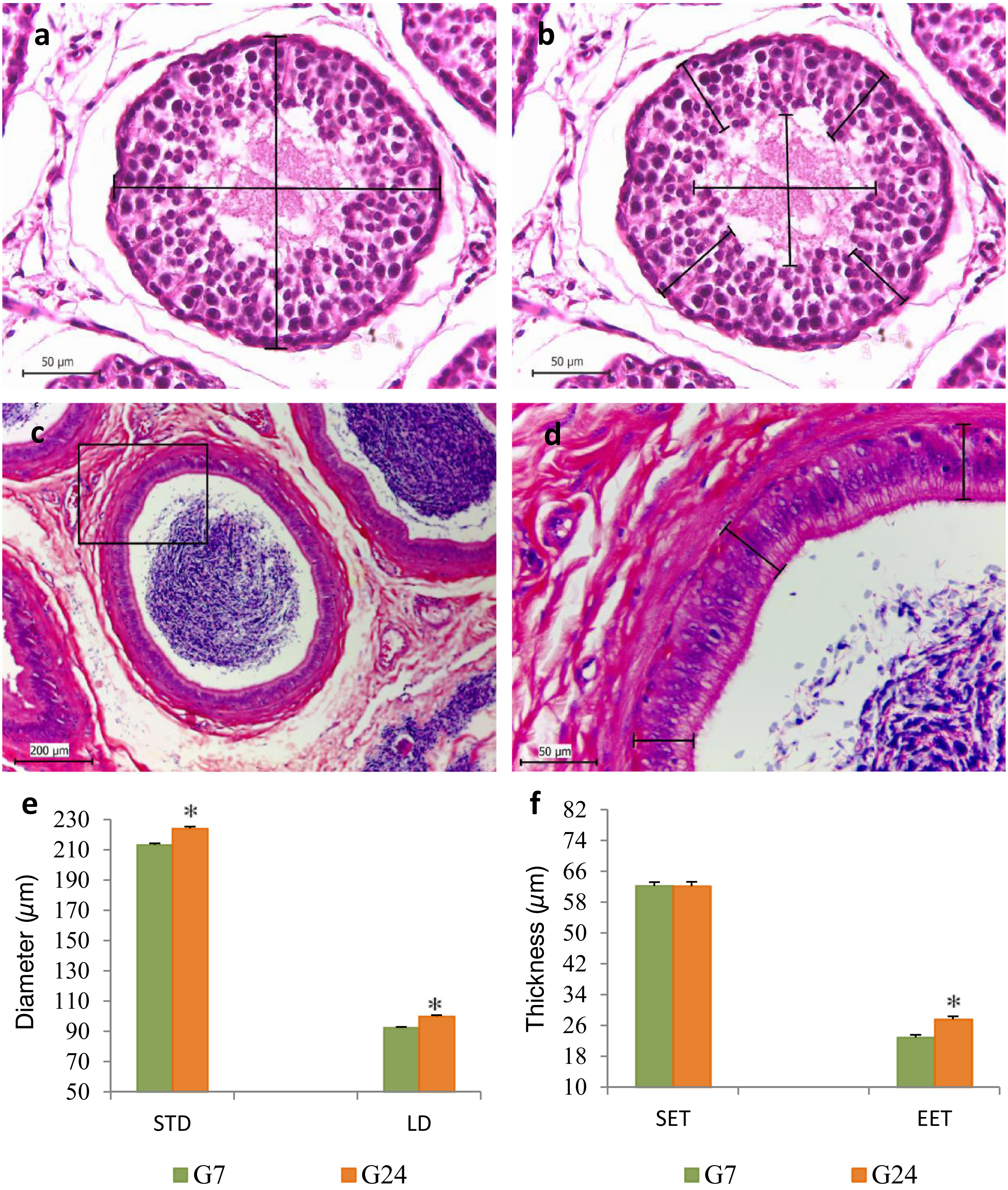

Fig. 5 Histomorphometric analysis of the testicles and tail of the epididymis of lambs fed different dietary energy levels (G7 and G24 = 7% and 24% increase in metabolizable energy, respectively, according to NRC 2007). Illustrative images of **a** evaluation of the seminiferous tubule diameter (STD), **b** of the lumen diameter (LD) and thickness of the seminiferous epithelium (SET), and **c**, **d** thickness of the epididymal epithelium (EET). **e**, **f** Means ± standard error per treatment (*$P < 0.05$). Hematoxylin-eosin staining; magnification of ×100 in **c** and of ×400 in **a**, **b**, and **d**

and Jorge 2010). In other species, the association between morphological development and sexual activity is used to identify more precocious animals (Garcia et al. 2010; Brito et al. 2012) in order to achieve a more profitable performance.

The variables of testicular biometry increased over the experimental period. This could benefit the earlier selection process of superior rams, which are valued due to the direct relation between scrotal circumference and reproductive precocity of the offspring (Toe et al. 2000). As testicular biometry and scrotal circumference are also dependent on factors such as body size and body condition score (Bailey et al. 1998), the influence of diet on reproductive traits is even more important, since the lambs studied were young animals and able to present high body growth rates. Since animals that received more energy had better response in testicular development, revealed by the higher values of scrotal circumference and testicular biometry, it can be assumed they show improvement in certain reproductive characteristics, or even better sexual performance, which are relevant criteria for using males in reproductive programs (Toe et al. 2000). In addition, rams fed a high level energy presented higher body condition score and probably would be more prone to fertilize ewes during a breeding season. This is a period in which males are extremely required to mate and consequently the energetic reserves are mobilized. A desired condition score can also favor the expression of puberty and the libido, as well the sperm quality (Alkassf et al. 1982; Valasi et al. 2012). Since the scrotal circumference of Morada Nova yearling rams varies from 27.5 to 33.6 cm (Sousa et al. 2014; Kahwage et al. 2017), higher precocity was observed in this aspect for animals that received higher energetic content, which presented 28.0 cm of scrotal circumference at an approximate age of 7 months. At least for this feature, the nutritional management seems to contribute for anticipating the pubertal characteristics by 3 to 5 months.

Testosterone is an essential hormone for the development of male secondary sexual characteristics (Galea et al. 2006). Its concentration was not influenced by the dietary energy levels between treatments. It is possible that the additional energy supply did not take place at a sufficient level to modify the steroidogenesis of the animals (Santos et al. 2000). In addition, the absence of the expressive endocrine effect may have occurred because since the first analysis the animals presented higher testosterone production than the expected minimum concentration for pubertal sheep, which is 1.4 ng/mL (Schanbacher et al. 1974). Similarly, Selvaraju et al. (2012) found that the supply of diets with a high (+ 20%) or low (− 20%) level of energy did not cause differences in testosterone concentrations in rams. It is possible that starting a supplementary strategy at early ages might have a significant effect on testosterone levels.

The success rate of semen collections was not influenced by the dietetic energy provided. It probably occurred because during the seminal collections, the animals were already able to perform gamete emission (Hafez and Hafez 2004) and the semen collection was favored by the method adopted. The seminal quality did not show significant changes between treatments for most of the analyzed variables. However, the groups differed in the incidence of minor sperm defects and total defects, with a significant reduction in sperm abnormalities of the animals in G24. This finding was favorable to the seminal quality of the animals that received the greatest amount of energy in the diet, which also demonstrated an increase in the thickness of the epididymal epithelium. A parietal enlargement of the epididymal ducts may have increased its functionality, mainly because this organ plays an important role in sperm maturation, which would positively influence the morphology of spermatozoa (Blom 1983). Sperm maturation occurs during epididymal transit, where cells acquire progressive motility and the ability to fertilize oocytes. It involves the interaction of spermatozoa with proteins that are synthesized and secreted in a region-dependent mode from the epididymal epithelium (Cornwall 2009). Although the epididymal tail is the place where sperm maturation occurs, with changes in morphology and motility enhancement (Zhou et al. 2004), increases in sperm motility were not observed with the highest energy intake.

Nutrition also has an effect on testicular development, which may affect the anatomical structure of the seminiferous tubules (Hotzel et al. 1998). This effect was emphatically observed in animals fed a higher-energy diet, which showed increased diameters of the seminiferous tubules and the lumen. This confirms that energy is a determinant factor in the general reproductive aspect, information corroborated by previous results that demonstrated that the energy restriction decreases the size of the seminiferous tubule and seminiferous lumen diameter of rams (Pang et al. 2017). This may possibly occur because the increase in diameter results in a large part of the mitotic activity of spermatogonia and Sertoli cells. It consequently increases germ cell activity and promotes spermatogenic cell proliferation and differentiation, which are mainly responsible for the increase of the seminiferous tubule (França et al. 2000; Herrera-Alarcón et al. 2007). However, the appearance of the tubular lumen is a consequence of increased secretion and accumulation of fluid produced by Sertoli cells, a stage that is part of the development process of the seminiferous epithelium (Russell et al. 1989).

Conclusions

The higher level of energy in the diet resulted in more pronounced body development, mainly after the fifth week of treatment. Animals that received a high-energy diet also had higher scrotal circumference and testicular biometry, as well as increase in the diameters of the seminiferous tubule and the thickness of the epididymal epithelium. However, the energy difference in the diets did not influence the concentration of testosterone in the lambs but favored the seminal quality by reducing the incidence of minor sperm defects, raising the index of normal sperm morphology. Thus, it is evident that with a higher-energy diet, lambs of the Morada Nova breed can express greater productive efficiency, with reproductive attributes of interest, which may contribute to enhance the genotype and favor the use of this genetic resource in sheep production systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. All co-authors participated in the execution of the research, in the writing of this article, and they agree with this final version.

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