MORPHOLOGICAL EVALUATION OF THE TESTICLES OF YOUNG SANTA INÊS RAMS SUBMITTED TO DIFFERENT REGIMES OF PROTEIN SUPPLEMENTATION AND DRENCHING

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ABSTRACT

The present study investigated the effect of protein supplementation and parasite load in relation to testicular morphology, in young Santa Inês male sheep. Twenty-four male lambs, non-castrated, were distributed in four treatments: HPd (dewormed animals + diet with high protein), HPn (animals non dewormed + diet with high protein), LPd (dewormed animals + diet with low protein), LPn (animals not dewormed + diet with low protein) during eight months. At the end of the experiment histological cuts of testicles were taken and cell count analyzed by computer and the program IMAGE PRO-PLUS was used for evaluation of measurements of the seminiferous tubules. The following results were found: LPn presented larger number of spermatogonias, but a reduced number of the other cells parameters (Sertoli cells, spermatocytes, spermatids and spermatozoa) and smaller diameter and circumference of the seminiferous tubules and shorter seminiferous epithelium when compared to other treatments. There was a significant correlation among tubules diameter of the epithelium height and tubules circumference. Correlations between lumen diameter and lumen circumference, as well as epithelium height and tubules circumference also presented high and positive correlations ($r>0.70$). When the external testis parameters were compared with internal parameters no significant correlation was observed ($r<0.50$). In conclusion, high protein supplementation positively influences spermatogenesis, and non-deworming associated with a poor protein diet worsens almost all reproductive parameters of young Santa Inês rams.

KEY WORDS: Morphology, nutrition, ovine, parasites, testicular morphometrics.

RESUMO

AVALIAÇÃO MORFOLÓGICA DOS TESTÍCULOS DE OVINOS JOVENS SANTA INÊS SUBMETIDOS A DIFERENTES REGIMES DE SUPLEMENTAÇÃO ALIMENTAR E VERMIFUGAÇÃO

O presente trabalho objetivou a investigação do efeito da suplementação protéica e carga parasitária em relação à morfometria testicular, em carneiros jovens da raça Santa Inês. Utilizaram-se 24 cordeiros, inteiros, distribuídos em quatro tratamentos: HPd (animais vermifugados + concentrado com alta proteína), HPn (animais não-vermifugados + concentrado com alta proteína), LPd (animais vermifugados + concentrado com baixa proteína), LPn (animais não vermifugados + concentrado com baixa proteína) por oito meses. Realizaram-se cortes histológicos dos testículos após abate e análises em computador para contagem de células, mediante o programa IMAGE PRO-PLUS, para avaliação das mensurações testiculares. O tratamento de baixa proteína sem vermifugação (BPn)

apresentou número maior de espermatogônias, porém número reduzido para células de Sertoli, espermatócito, espermátide e espermatozóide; o tratamento BPn apresentou menor diâmetro e circunferência do túbulo e altura do epitélio seminífero quando comparado aos demais tratamentos; houve correlação significativa entre o diâmetro do túbulo com altura do epitélio e circunferência do túbulo. As correlações entre o diâmetro do lúmen e a circunferência do lúmen, bem como entre a altura do epitélio e a circunferência do túbulo, também apresentaram correlações altas e positivas (P>0,70); comparando-se os parâmetros testiculares externos com os internos, observa-se que não houve correlação significativa (P<0,50). Conclui-se que a alta proteína influencia positivamente a espermatogênese, e a não-vermifugação associada a uma dieta pobre em proteínas pioram substancialmente a maioria dos parâmetros reprodutivos de carneiros jovens Santa Inês.

PALAVRAS-CHAVES: Morfometria testicular, morfologia, nutrição, ovino, parasitas.

INTRODUCTION

Demand for sheep meat has grown strongly in the last years in Central Brazil, requiring greater efficiency of the sector. The Distrito Federal (DF) stands out among the states in the Brazilian Center-west for having not only the largest sheep concentration (0.48 animal/km^2), but also the largest per capita market for sheep meat (MCMANUS et al., 2002). In spite of the increase in flock efficiency, there is a need to improve carcass quality and reproductive performance. The reproductive efficiency of the male has been little studied and is known to be affected by several factors, nutrition and health among them.

It has already been established that nutrition affects testicular size (MOULE, 1963; SALAMON, 1964; SETCHELL et al., 1965; PARKER & THWAITES, 1972; BRADEN et al., 1974; ALKASS et al, 1982; MASTERS & FELS, 1984; MARTIN et al., 1994), spermatozoa production (OLDHAM et al., 1978; CAMERON et al., 1988) and testicular morphometrics (BIELLI et al., 1999; MOREIRA et al., 2001; MOTA et al., 2001; SALHAB et al., 2001) in rams. However, only a few studies demonstrate how the volume of the seminiferous epithelium and the diameter of the seminiferous tubules are affected (SETCHELl et al., 1965; OLDHAM et al., 1978; HÖTZEl et al., 1998). Besides nutrition, another factor that affects the development of sheep breeding is worm load, requiring the adaptation of nutritional demands of animals with parasitic infection (VELOSO, 2002).

This work aims to evaluate the effects of protein supplementation and infection of endoparasites in the morphometric characteristics of testis in Santa Inês rams, at pasture conditions in Central Brazil.

MATERIAL AND METHODS

Animals and treatments

Experiments were developed in the Sheep Center at University of Brasília. Twenty-four Santa Inês male lambs with 4 months of age and medium (±SD) initial weight of 24.5 ± 2.88 Kg were used in the experiment. They were randomly distributed in four treatment groups (n=6 animals/treatment): HPd (dewormed animals + high protein supplementation), HPn (non-dewormed animals + high protein supplementation), LPd (dewormed animals + low protein supplementation), LPn (non-dewormed animals + low protein supplementation).

Experiments began with all animals under the same parasitic conditions, all dewormed and, starting from there only the dewormed groups received monthly anti-helmintic treatment (levamizol phosphate – Ripercol Fort Dodge Saúde Animal Ltda, and a pirantel, oxantel and praziquantel solution, formulated for the experiment by Agribrands do Brasil Ltda) in the dosage recommended by the manufacturer.

Animals were maintained in a single picket of 5 ha, on a pasture of Andropogon gayanus, which was analyzed every month according to the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1995). They were supplemented with one of two different concentrate mixtures, one with high protein (30%

soybean meal, 20% wheat meal, 46% corn and 4% minerals and vitamins) and other with low protein (10% soybean meal, 10% wheat meal, 76% corn and 4% minerals and vitamins), according to the experimental group. The diet was calculated based on the nutritional demands established by AFRC (1993). The animals received the concentrate always at late afternoon, 300g/animal/day during the wet season, and 500g/animal/day during the dry season. Animal groups were fed and dosed individually. The animals had free access to water. Pasture analysis and diet composition are provided in Table 1.

The climate in the region is AW by the Köppen classification, with a mean annual temperature of 21.1 °C, varying between an absolute daily minimum and maximum of 16° and 34°, respectively. Annual mean precipitation is 1578.5mm and mean annual relative humidity of the air is 68%. The climate is characterized by two well defined seasons, rainy, in which almost all precipitation occurs (from October to April) and dry (from May to September).

TABLE 1. Bromatological composition of the high and low protein concentrates and pasture (mean ± SD) based on dry matter.

Constituents	Concentrate		Pasture	
	High protein	Low protein	Wet season	Dry season
Dry matter, %	88.7	88.1	23.1 ± 7.6	46.1 ± 5.6
Crude protein, %	19.2	11.5	9.2 ± 2.9	4.9 ± 0.1
Brute fiber, %	3.6	2.6	25.1 ± 3.7	28.3 ± 2.7
Ether extract, %	2.7	3.0	1.8 ± 0.8	1.8 ± 0.3
Ash, %	6.3	4.3	5.9 ± 1.1	6.3 ± 0.6
Metabolizable energy (MJ/KgDM)*	13.2	12.8	-	-

*Calculated by AFRC (1993)

Every month during the experiment, the animals were weighted and fecal egg counting was performed according to the modified McMaster technique (WHITLOCK, 1948). After 8½ months on the experiment, animals were slaughtered following a 24-hour fast. Body weight was determined before the fasting period.

Testicle measurements

During fasting period, biometrical measurements of in situ testis characteristics (scrotal circumference – SC, testicular length – TL and width – TW) were taken on each animal. After slaughter, the animals testicles were collected and the real testis volume (TV) was measured through water displacement.

Histological analysis and image processing

After slaughter and in vitro measurements, both testicles were fragmented and two samples per testicle were randomly taken and fixed in Bouin for 6hs. After fixation, the samples were dehydrated, clarified and embedded in paraffin wax. Specimens were cut at 3µm thickness and stained with haematoxylin and eosin. For each block, 2 slides were prepared and analyzed.

Digital capture of the images was performed in an AxioSkop Microscope (Zeiss) coupled with a colored digital CCD camera (Sony, I model DXC-107A) and a computer with a digital capture Pixel View Play TV plate (640 X 480 pixels). For each slide, 5 seminiferous tubules randomly chosen were analyzed, and the following aspects were observed: number of Sertoli cells, number of cells of the spermatogenetic lineage, presence of spermatozoa, diameter and circumference of the tubule and its lumen and height of the seminiferous epithelium. Measurements were carried out with the aid of a morphometric analyses program (IMAGE-FOR PLUS, 1999, version Measured Cybernetics, L.P).

Statistical Analysis

Data were analyzed using GLM (General Model Linear), CORR (Correlation) of SAS (1990). The analysis of variance evaluated the diet and deworming effects and their interactions for the morphological characteristics of the testicles: diameter and circumference of the seminiferous tubules, diameter and circumference of the lumen of the tubules, height of the seminiferous epithelium, number of Sertoli cells, spermatogonias, spermatocytes, spermatids and spermatozoa. Live weight was included as a co-variable and was not significant for our trait. No statistical differences were found among the measurements of the left and right testicles. The data was transformed by log (x) when necessary.

RESULTS AND DISCUSSION

At the beginning of the experiment, the animals presented similar body weight (P>0.05). From the second month on, animals receiving HP diet had higher weight gain than those receiving LP diets. However, with the beginning of dry season (May), HPn group started loosing weight, and there was no significant difference (P>0.05) between this group and LPd and LPn groups. Also, there was no difference (P>0.05) between LPd and LPn group animals concerning body weight during all the experiment. The final weight of animals from HPd, HPn, LPd and LPn was 44.08, 37.44, 36.37, and 35.62kg, respectively (Figure 1).

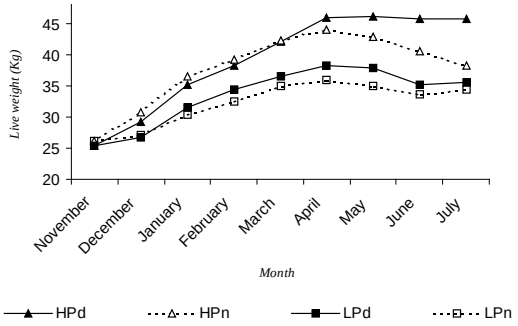

FIGURE 1. Weight variation in Santa Inês rams receiving high and low protein diets and with or without anti-helmintic treatment, from November, 2000 to July, 2001.

At the end of the experiment, dewormed animals receiving HP and LP diets presented, respectively, 14.29 and 107.14 eggs per g on average on the fecal egg counting and non-dewormed animals from HP and LP showed 2292.86 and 5550 eggs per g, respectively.

Table 2 shows the morphometrics of the seminiferous tubules and testicles. Photomicrography of seminiferous tubules of animals from the LPn and HPd groups are shown in Figures 2 and 3, respectively. A closer view of the seminiferous tubule of an animal from HPd group is shown in Figure 4.

TABLE 2. Morphometric measurements (average) of seminiferous tubule (in µm) and testis (in cm) measurements in young Santa Inês sheep on different treatments.

Treatment	Tubule diameter	Tubule circumf.	Lumen diameter	Lumen circumf.	Epithelium height	Scrotal circumf.	Testicular length	Testicular width	Testicular volume
HPd	185.91^{a}	28086.66^{a}	76.89^{a}	4779.05^{a}	55.17^{a}	28.70^{a}	8.82^{a}	5.86^{a}	514.0^{a}
HPn	178.94^{a}	26246.34^{a}	76.83^{a}	5011.48^{ab}	52.50^{a}	26.78^{a}	8.08^{ab}	4.84^{ab}	392.0^{b}
LPd	182.95^{a}	27209.57^{a}	89.59^{b}	6060.29^{b}	50.89^{ab}	24.56^{b}	7.51^{b}	4.50^{b}	305.0^{b}
LPn	157.59^{b}	20967.26^{b}	73.88^{a}	4360.10^{a}	44.61^{b}	25.77^{ab}	7.62^{b}	4.71^{b}	328.6^{b}

^{ab} Means within columns followed by different letters differ at P<0.05

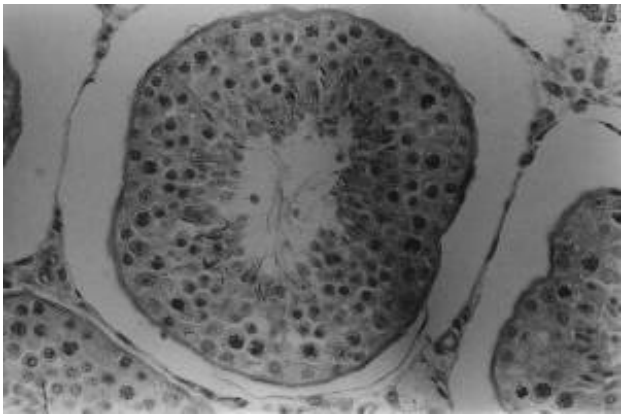

FIGURE 2. Histological section of seminiferous tubule from LPn group. 450x. Barr = 20µm

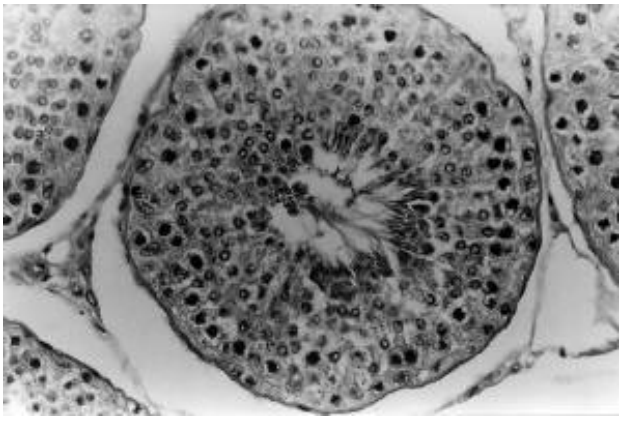

FIGURE 3. Histological section of seminiferous tubule from HPd group. 450x. Barr = 20µm.

FIGURE 4. Histological section of seminiferous tubule from HPd group. Arrow-head shows the seminiferous tubule basement membrane; big arrows show spermatocytes I; small arrows show spermatocytes II; * seminiferous tubule lumen (observe spermatozoa in the lumen); S: Sertoli cell. 1100x. Barr = 10µm.

The LPn group had a smaller diameter and circumference of the tubule when compared to the other treatments. Moreover, the height of the seminiferous epithelium was smaller in animals from LPn than in animals from HPd and HPn. On the other hand, the diameter and circumference of the lumen was larger in LPd compared to the other groups.

HÖTZEL et al. (1998), working with Merino sheep, presented similar results, showing that there was an increase in length and diameter of the seminiferous tubules when animals were fed with high protein diet. Similar results were also presented by BIELLI et al. (1999; 2000) whom described that Corriedale sheep at pasture and fed with concentrate had larger tubular diameter and circumference. In the present work, it was also observed a significant increase in the seminiferous tubule lumen diameter in animals from LPd in relation to the other treatment groups. This agrees with Hötzel et al. (1998), which demonstrated that lumen diameter was larger in Merino sheep fed with low protein diets. Although in the present work the lumen diameter in LPn treatment was not larger than in the other treatments, it was a reflex of the small diameter and circumference of the tubule. Our results suggest that animals fed with low protein diets (LPd and especially LPn) presented the most unfavorable morphometric condition.

Based on the results, it was possible to observe that a diet with low protein associated with no deworming of the animals resulted in more evident histological alterations, emphasizing an interaction effect between diet protein levels and deworming. Although it can be used as an energy source, proteins are also used for specific needs in the body, such as: growth, secretions, renewal of tissues and cells and gamete production (D'ARCE, 1995).

Table 3 shows the mean number of Sertoli cells and spermatogenetic cells in the seminiferous tubules of animals in each treatment. It can be observed that animals from LPn presented lower numbers of Sertoli cells, spermatocytes, spermatids and spermatozoa, while it presented higher numbers of spermatogonia when compa-

red to the other treatments. The number of Sertoli cells was significantly lower in LPn while the number of spermatogonia was significantly higher than in the other treatments. Nonetheless, the number of spermatocytes was only significantly different from that observed in animals from HPd. Spermatid numbers were significantly lower in animals from HPd and HPn. LPd and LPn showed a significantly reduced number of spermatozoa when compared to HPd and HPn.

TABLE 3. Effect of treatments on the number of cells per seminiferous tubule in young Santa Inês sheep.

Treatment	Sertoli	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa
HPd	10.80[a]	7.09[a]	28.60[a]	52.18[a]	2.05[a]
HPn	10.62[a]	7.81[a]	26.12[ab]	51.36[a]	2.06[a]
LPd	9.78[a]	7.87[a]	25.12[ab]	46.67[ab]	1.57[b]
LPn	8.21[b]	9.47[b]	24.04[b]	39.42[b]	1.64[b]

[ab] Means within columns followed by different letters differ at P<0.05

A smaller number of Sertoli cells was observed in animals from LPn. Other researchers also observed similar results in animals fed with low protein diets (HÖTZEL et al., 1998; BIELLI et al., 2001). YANG et al. (1990) and CUNNINGHAM (1999) affirmed that the population of Sertoli cells is already established before puberty, when those cells lose their ability to multiply by mitosis. A poor nutritional condition before puberty, similar to what happened in the present work, may affect the number of these cells. In their study BRONGNIART et al. (1985) also showed that when lambs were submitted to food restrictions from birth till puberty histology of testicles showed a reduced number of Sertoli cells when compared to control animals. In mice, it was also observed that, after food restriction of pregnant females, there were an accentuated testicular regression and increase of apoptosis of the testis cells of born nestlings (MOTA et al., 2001). MOREOVER et al. (1998) demonstrated that, in adult Merino sheep, there was a decreased number of Sertoli cells in the group of animals that had a poor protein diet in relation to the high protein group. According to CUNNINGHAM (1999) an increase in the number of Sertoli cells is unlikely to happen in physiological conditions, but the referred works suggest that a severe lack of nutrients could promote a decrease in the number of these cells.

The results of HÖTZEL et al. (1998) showed that there was an increase in the total number of spermatogenic cells when the animals were fed with concentrate. In the present study, the number of spermatocytes was significantly higher in HPd in relation to LPn. Also, a greater number of spermatids was observed in HPd and HPn than in LPd and LPn. However, the number of spermatogonia was higher in LPn animal than in the other groups. The result of the spermatocytes is in agreement with HÖTZEL et al. (1998). Additionally, spermatids results are in accordance to the findings of other authors (HÖTZEL et al., 1998; BIELLI et al., 1999; 2000). A balanced feeding probably positively affects the spermatogenesis. Hypothetically, in an animal with unfavorable protein diet, the spermatogenesis process would be slowed down and, therefore the spermatogonia numbers would be higher, while the spermatocyte and spermatid numbers would be lower. Inversely, in an animal with balanced protein diet, the spermatogonia progression to spermatocyte and spermatid would have increased and then the spermatogonia numbers would be lowered. Moreover, the number of spermatozoa was smaller in animals supplemented with low protein. This reinforces that the nutritional balance of the animal affects the spermatogenesis.

The correlations among the diameter of the tubule with tubule circumference and epithelium height were high and positive (r>0.72-0.95), but for the other characteristics it was medium to low (r<0.50). The correlations for all the other parameters presented medium and low correlation (r<0.50). HÖTZEL et al. (1998) demonstrated that there is a strong correlation among the production of spermatozoa with the diameter and size of the seminiferous tubule and with the amount of seminiferous tissue. In the present work, larger tubule diameter and higher seminiferous epithelium were observed in animals fed with high protein diets (HPd and HPn), which might reflect on the sperm production.

Regarding testicle measurements, animals from LPd group showed a smaller scrotal circumference when compared to animals from HP treatments. In other testicle characteristics (length, width and volume), HPd was significantly higher than LP groups. It was also higher than HPn in real testicle volume (Table 2). External testis parameters (scrotal circumference, testicle length and width and testicle volume) were highly correlated with body weight (r>0.70). However, correlations between histological and biometric parameters of the testis were always low (r<0.50) and not significant. Scrotal circumference is often used to presume semen concentration (SANTANA et al., 2001), but usually animals used for reproduction are well fed, which is not the case here. The animals in this work were maintained in alimentary regimes similar to that found on farms in the central area of Brazil. BIELLI et al. (1999, 2000) affirmed that testicular volume has a high correlation with Sertoli cell and spermatid numbers, which was not observed in the present study. Here, the increase in the external parameters did not reflect in a larger production of cells from spermatogenic lineage or an increase in the size of seminiferous tubules. This can be explained by the increase in the amount of interstitial tissue, instead of tubular tissue. This is reinforced by HÖTZEL et al. (1998), which affirmed that Merino sheep fed with poor protein diets presented more interstitial tissue than those fed with high protein diets.

CONCLUSIONS

Lack of drenching associated with a poor protein diet worsens most of the reproductive parameters in young males of Santa Inês breed. Supplementation with high protein influences positively spermatogenesis, and some times even compensates the lack of drenching. Large external testis parameters do not mean high production of cells from the spermatogenic lineage. Therefore semen evaluation is important, especially in animals in poor feeding regimes.

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REFERENCES

AGRICULTURAL AND FOOD RESEARCH COUNCIL (AFRC). **Energy and requirement of ruminants**. Advisory manual prepared by AFRC technical Committee on responses to nutrients. CAB International, Wallingford, 1993. 159 p.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. **Official methods of analysis of the AOAC.** v.1. 16th edition. Arlington: AOAC International, 1995. p. 4/1-4/30.

ALKASS, J.E.; BRYANT, M.J.; WALTON, J.S. Some effects of level of feeding and body condition upon sperm production and gonadotrophin concentrations in the ram. **Animal Production**, v. 34, p.265-277, 1982.

BIELLI, A.; GASTEL, M.T.; PEDRANA, G.; MORAÑA, A.; CASTRILLEJO, A.; LUNDEHEIM, N.; FORSBERG, M.; RODRIGUEZ-MARTINEZ, H. Influence of pre- and post-pubertal grazing regimes on adult testicular morphology in extensively reared Corriedale rams. **Animal Reproduction Science**, v. 58, p.73-86, 2000.

BIELLI, A.; KATZ, H.; PEDRANA, G.; GASTEL, M.T.; MORAÑA, A.; CASTRILLEJO, A.; LUNDEHEIM, N., FORSBERG, M.; RODRIGUEZ-MARTINEZ, H. Nutritional management during fetal and postnatal life, and the influence on testicular stereology and Sertoli cell numbers in Corriedale rams. **Small Ruminant Research**, v. 40, p. 63-71, 2001.

BIELLI, A.; PEDRANA, G.; GASTEL, M.T.; CASTRILLEJO, A.; MORAÑA, A., LUNDEHEIM, N.; FORSBERG, M.; RODRIGUEZ-MARTINEZ, H. Influence of grazing management on the seasonal change in testicular morphology in Corriedale rams. **Animal Reproduction Science**, v. 56, p.93-105, 1999.

BRADEN, A.W.H.; TURNBULL, K.E.; MATTNER, P.E.; MOULE, G.R. Effect of protein and energy content of the diet on the rate of sperm production in rams. **Australiana Journal of Biological Science**, **v.** 27, p.67-73, 1974.

BRONGNIART, I.; THERIEZ, M.; PERREAU, C.; HOCHEREAU-DE REVIERS, M.T. Testis parameters, LH and testosterone secretion in underfed lambs. **Proc.** III **Internat. Cong. And. J. And**., supl. 6, p. 64, 1985.

CAMERON, A.W.N.; MURPHY, P.M.; OLDHAM, C.M. Nutrition of rams and output of spermatozoa. **Proc. Aust. Soc. Anim. Prod**., v. 17, p.162-165, 1988.

CUNNINGHAM, J.G. Fisiologia reprodutiva do macho. In: CUNNINGHAM, J.G. (Ed.). **Tratado de fisiologia veterinária.** 3. ed. Rio de Janeiro: Guanabara Koogan, 1999. p. 432-439.

D'ARCE, R.D. Fisiologia da digestão. In: PEIXOTO, A. (Ed.). **Nutrição de bovinos.** 3. ed**.** Piracicaba: FEALQ, 1995. p.1-12, 1995.

HAFEZ, E.S.E.; HAFEZ, B. Foliculogênese, maturação do ovo e ovulação. In: HAFEZ, E. S. E.; HAKEZ, B. (Ed.). **Reprodução animal.** 7. ed. São Paulo: Editora Manole, 2004. p.69-82.

HÖTZEL, M.J.; MARKEY, C.M.; WALKDEN-BROWN, S.W.; BLACKBERRY, M.A.; MARTIN, G.B. Morphometric and endocrine analyses of the effects of nutrition on the testis of mature Merino rams. **Journal of Reproduction and Fertility**, v. 113, p.217-230, 1998.

JOLIFFE, I.T. **Principal component analysis**. New York: Springer-Verlag, 1986.

Martin, G.B.; Walkden-Brown, S.W.; Blackberry, M.A. Effects of nutrition on testicular size and the plasma concentrations of gonadotrophins, testosterone and inhibin in mature male sheep. **Journal of Reproduction and Fertility**, v. 101, p. 121-128, 1994.

MASTERS, D.G.; FELS, H.E. Seasonal changes in the testicular size of grazing rams. **Proc. Aust. Soc. Anim. Prod.,** v. 15, p. 444-447, 1984.

MCMANUS, C., LOUVANDINI, H., LEITE, G., COUTO, F.D., MEDEIROS, J. X. **Avaliação da cadeia produtiva de caprinos e ovinos no DF**. Brasília: Fundação de Apoio à Pesquisa do Distrito Federal, 2002. 83 p.

MOULE, G.R. Postpubertal nutrition and reproduction by the male. **Australian Veterinary Journal**, v. 39, p.299-304, 1963.

MOREIRA, E.P.; MOURA, A.A.A.; ARAÚJO A.A. Efeitos da insulação escrotal sobre a biometria testicular e parâmetros seminais em carneiros da raça Santa Inês criados no Ceará. **Revista Brasileira de Zootecnia**, v. 30, p. 1704-1711, 2001.

MOTA, E.C.; SANTOS, A.M.; TOSTE, F.P.; SAMPAIO, F.J.B.; RAMOS, C.F. Effects of malnutrition in the testis. **Brazilian Journal of Urology**, v. 27, p.500-506, 2001.

OLDHAM, C.M., ADAMS, N.R., GHERARDI, P.B., LINDSAY, D.R., MACKINTOSH, J.B. The influence of level of feed intake on sperm-producing capacity of testicular tissue in the ram. **Australian Journal of Agriccultural Research**, v. 29, p. 173-179, 1978.

PARKER, G.V.;THWAITES, C.J. The effects of undernutrition on libido and semen quality in adult Merino rams. **Australian Journal of Agriccultural Research**, v. 23, p. 109-115, 1972.

SALAMON, S. The effect of nutritional regimen on the potential semen production of rams. **Australian Journal of Agriccultural Research,** v. 15, p. 645-656, 1964.

SALHAB, S.A.; ZARKAWI, M.; WARDEH, M.F.; ALMASRI, M.R.; KASSEM, R. Development of testicular dimensions and size and their relationship to age body weight and parental size in growing Awassi ram lambs. **Small Ruminant Research**, v. 40, p. 187-191, 2001.

SANTANA, A.F. De; COSTA, G.B.; FONSECA, L.F. Avaliação da circunferência escrotal como critério de seleção de machos jovens da raça Santa Inês. **Revista Brasileira de Saúde e Produção Animal**, v.1 , p.27-30, 2001.

SAS INSTITUTE. **Statistical Analysis System**: user's guide. 4.th ed. Cary, 1990. 890 p.

SETCHELL, B.P.; WAITES, G.M.H.; LINDNER, H.R. Effect of under nutrition on testicular blood flow and metabolism and the output of testosterone in the ram. **Journal of Reproduction and Fertility**, v. 9, p.149-162, 1965.

Veloso, C.F.M. **Efeitos da suplementação protéica na verminose e nas características de carcaça de ovinos da raça Santa Inês mantidos a pasto**. Brasília, 2002, 50 f. (Master of Science Dissertation) – Universidade de Brasília.

WITHLOCK, J.H. Some modifications of the McMaster helminth egg counting technique and apparatus. **J. Coun. Scient. and Ind. Res**., v. 21, p.177-180, 1948.

YANG, Z.W.; WREFORD, N.G.; KRETSER, D.M. A quantitative study of spermatogenesis in the developing rat testis. **Biology and Reprodudction**, v. 43, p.629-635, 1990.

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