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Effect of dietary energy intake and somatotropin administration after weaning on growth rate and semen characteristics of Granadina goat bucks

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Abstract: Twenty-eight growing Granadina goat bucks, divided into 4 groups of 7, in a 2 × 2 factorial arranged design, were used to study the effect of post-weaning levels of dietary energy and administration of recombinant bovine somatotropin (rbST) on growth and semen characteristics. Every 14 days, bucks were offered sustenance at rates of either 1.0 (standard diet, 2.36 Mcal EM kg^{-1}; 15% CP) or 1.25 (increased diet, 2.95 Mcal EM kg^{-1}; 18% CP) times the requirements for growth energy and protein, for a total of 99 days and in combination with or without subcutaneous administration of 125 mg rbST. The average daily gains (ADG) were greater ($P < 0.01$) in bucks fed the high energy diet (133 ± 25 vs. 111 ± 23 g day^{-1}) and the ADG were greater ($P < 0.05$) in the bucks treated with rbST than in the untreated bucks (130 ± 28 vs. 114 ± 23 g day^{-1}). The percentage of live sperm cells was not different between bucks fed increased diet of NRC recommendations and bucks fed standard diet. Similarly, the sperm output of bucks on the increased diet did not differ from that of bucks on the standard diet (2282 ± 1137 vs. 1946 ± 529 × 10^6/mL). Semen volume (0.51 ± 0.29 vs. 0.55 ± 0.28 mL), sperm concentration (2210 ± 1139 vs. 2055 ± 656 × 10^6/mL), total sperm cells (1233 ± 962 vs. 1014 ± 572 ×x 10^6), and motile sperm cells (67.1 ± 14.5 vs. 60.9 ± 19.3) were not affected by rbST. No significant differences due to dietary and hormonal treatments were observed in scrotal circumference. Dietary and hormonal treatments had no effect on either serum concentration of particular metabolites, except cholesterol. Results from this study indicate that both high energy diets and chronic application of rbST enhanced the growth performance of young Granadina goat bucks; nevertheless, none of these effects altered the final scrotal circumference, particular blood metabolites, and sperm output.

Key words: Growth, scrotal circumference, blood metabolites, sperm

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Introduction

The importance of using only highly fertile bucks in breeding programs is often neglected, especially in goat operations under extensive conditions, in arid zones of the world. In northern Mexico, most range flocks average 3-4 males per 100 does; however, because of the high serving capacity of bucks (1) fewer bucks may be employed. However, as no breeding soundness tests are applied to bucks before breeding, this high proportion of goat bucks compensates for subfertile or underfed bucks and usually, satisfactory pregnancy rates are achieved, even under extremely dry conditions (2). Goat producers will be rewarded for applying a few simple techniques to improve the breeding ability of their bucks, such as an adequate nutrition, because the interrelationship between energy intake and reproductive performance in adult goat bucks has been demonstrated (3). Supplementary feeding to goat bucks increases sperm quantity and quality as compared with unsuplemented animals (4,5). On the other hand, administration of exogenous somatotropin is one biotechnology that increases meat output per unit of feed resource input in ruminants (6). Additionally, growth hormone (GH) has a role on the regulation of testicular function in some species (stallion: 7, rats: 8, bulls: 9, and humans: 10). There is substantial evidence that GH, besides its effects on somatic growth and differentiation, modulates gonadal steroid synthesis and gametogenesis (11).

It is unknown if the effects of GH on nutrient partitioning are testis function-specific in goats. Moreover, body composition, which affects potential nutrient partitioning properties of rbST, varies among animal types. Therefore, the primary goal of the current experiment was to test the hypothesis that rbST and dietary energy level would increase semen quality in young Granadina goat bucks during the breeding season; live weight and blood metabolite responsiveness to somatotropin and dietary energy level were also studied as a secondary goal.

Materials and methods

Animals and experimental procedure

This study was carried out over a period of 99 days, at the University Autonoma Agraria Antonio Narro, Saltillo, Mexico (25°22′N, 101°00′W; mean annual temperature 19.8 °C). A total of 28 Granadina goat bucks, initially averaging 14 ± 3.7 kg body weight (BW) and 2.9 ± 0.3 body condition score (5-point scale), were randomly allotted at weaning (12 week of age), to 4 experimental diets. Prior to weaning, the goat bucks were raised with their dams under pen conditions. The Granadina goat bucks used in the present study were much smaller than those raised in Spain, because for centuries, no selection effort for milk or meat production has been carried out in these goats, exploited under extensive conditions in harsh arid environments of Mexico.

The goat bucks were housed in outdoor pens with cement floors, in groups of 7 per pen, under ambient temperature and lighting. Prior to the initiation of experimental treatments, the growing goat bucks were ear tagged, treated for internal and external parasites (Ivomec, Merck, Rahway, NJ, USA), and vaccinated for protection against various clostridia (Bar-Vac CD/T®, Ignatius, MT, USA). The goats were weighed biweekly, without withdrawal of feed or water. Both initial and final weights were determined using the average of weights taken on 2 consecutive days. At the end of the feeding period, scrotal circumference was recorded by massaging the testes into the scrotum and using a flexible measuring tape to measure the circumference at the largest part of the scrotum. Moreover, during the last weighing, wither height was measured at the highest point on the buck's shoulder, immediately above the front legs with a graduated measuring stick.

The experiment was conducted from May to August (breeding season for this breed of goats in northern Mexico; 12) and consisted of a 10-day pretreatment period and 99 days of rbST treatment. Twice a day, the goats were given ad libitum access to diets prepared as a total mixed ration of either 1.0 (standard diet) or 1.25 (increased diet) times the maximum growth energy and protein requirements (13). Water and a free-choice mineral mix were available at all times. Troughs were cleaned out before feeding and refusals (around 20% of the feed offered) were discarded.

Bucks received rbST (Lactotropina®, Greenfield, IN, USA; 125 mg, s.c.) at the beginning of the experiment and every 14 days thereafter, until the end

of the experiment. The dosage of rbST administered was based on the amount given to commercial dairy cattle (1/4 of that administered to lactating dairy cows).

The experiment was a 2 × 2 factorial and was conducted as a randomized complete block design with 7 replicates. Semen was collected for the first time when all of the bucks were sexually active (average weight 26.3 ± 4.7 kg). After 5 days of sexual rest, the average of semen variables of 2 ejaculates collected at a 4-day interval were used for statistical analysis.

Blood and semen sampling

Latex condoms for men, previously washed to eliminate the lubricant, were used to collect the semen. Condoms were inserted into the vagina of 3 adult does in estrus, and the open part of the condom was attached to the vulva with 3M Transpore Surgical Tape 1" (St. Paul, MN, USA). The caudal part of the condoms was lubricated with a K-Y® gel (Johnson & Johnson, Mexico) prior to copulation. Bucks were joined with unrestrained does in a pen until they ejaculated into the condom. Immediately after collection, the semen was transferred to a graduate vial for volume determination to the nearest 0.1 mL.

Sperm concentration was determined using 0.025 mL of semen diluted with 0.5 mL of a fixative solution, containing 7% formaldehyde and 0.85% NaCl mixed in a 1:1 (vol/vol) ratio. The diluted semen was placed on a hemocytometer with the sperm counted in 5 squares of each of 2 chambers, and the concentration of sperm cells was calculated.

Because sperm cell viability was adversely affected by the condom material, an additional semen collection was made the next day by means of electrical stimulation, to determine sperm motility, percentage of live spermatozoa, and normal sperm cells. These ejaculates were immersed in a warm water bath at 37 °C until quality assessment. In order to evaluate forward progressive motility, semen samples were diluted in a sodium citrate solution, pH 6.7 to 6.9, transferred to a warmed slide (37 °C) within 3 min after collection, and observed under a phase-contrast microscope at 250×.

The total number of sperm was determined by multiplying sperm concentration by the volume of the ejaculate. Live sperm were evaluated by eosin/nigrosin stain exclusion. Briefly, a drop of stain was mixed with a drop of pure semen and extended on the slide. Two hundred spermatozoa were counted, and the unstained spermatozoa were determined as viable cells. In addition, the number of tailless sperm, those with coiled, bent, or shoehook tails, and those with cytoplasmic droplets were determined and the percentage of abnormal sperm was calculated in relation to the total sperm cells counted.

On the last day of the feeding trial (day 99), blood samples were obtained before the morning meal by jugular venipuncture into tubes without sodium heparin. The tubes were chilled immediately in an ice bath, transported to the laboratory, and centrifuged at 1500 × *g* and 4 °C for 20 min. Blood serums were separated and stored in 2 mL vials at –20 °C until analyses.

Serum metabolites were determined using spectrophotometric methods (Coleman Junior II). Serum total protein concentration was determined with a kit based on the bicinchoninic acid reagent, with bovine serum albumin as a protein standard (Pierce Chemical, Rockford, IL, USA). Glucose was assayed with kit 115-A, based on glucose oxidase, and urea was quantified using kit 640-A, based on urease (Sigma-Aldrich Co., St. Louis, MO, USA).

Table 1. Percentage composition (DM) of the experimental diets.

Ingredients	1.0[a]	1.25
Sorghum hay	52.0	36.0
Sorghum grain	16.0	24.0
Cotton seed meal	15.0	------
Wheat bran	------	2.0
Soybean meal	10.0	32.0
Molasses	5.0	------
Animal fat	------	5.0
Calcium carbonate	0.95	0.45
Phosphate dibasic	0.5	------
Trace minerals	0.05	0.05
Common salt	0.5	0.5
Nutrient content		
Crude protein (%)	15.0	18.75
ME (Mcal kg^{-1})	2.36	2.95

[a]Rates of 1.0 (standard diet) and 1.25 (increased diet) times the growth energy and protein requirements.

Creatinine was measured in serum using the QuantiChromTM Creatinine Assay Kit (DICT-500; BioAssay Systems, Hayward, CA, USA). Serum cholesterol was determined using the EnzyChromTM cholesterol assay kit (ECCH-100; BioAssay Systems; Hayward, CA, USA). Serum microminerals (Mg, Cu, and Zn) were determined by atomic absorption spectrophotometry (Perkin Elmer Instruments model 2380). Serum phosphorus was determined by a calorimetric method (14).

Statistical analysis

Data were initially analyzed as a 2 × 2 factorial arrangement of treatments and a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Motility of buck sperm, as well as percentage of live and normal sperm cells was subjected to arcsine transformation before analysis of variance to ensure normal distribution of the results obtained in percentages. Goat buck was used as the experimental unit; the model included energy level (rates of 1.0 and 1.25 nutrient requirement) and level of rbST (0 and 125 mg) and their interaction.

For all of the parameters analyzed, the interaction diet by level of rbST was nonsignificant ($P > 0.15$) and thus was dropped from the model, and data were pooled across energy level and rbST.

Results

Because there was no interaction between diet and rbST ($P > 0.15$), only the main effects of diet and rbST administration on growth are presented in Table 2. Administration of 125 mg rbST for 14 weeks increased the growth rate of young goat bucks by 14% ($P < 0.05$).

Dietary nutrients or rbST had no effect on height at withers (Table 2). Chronic administration of rbST did not affect the serum glucose concentration (Table 3). On the other hand, concentrations of serum cholesterol were influenced ($P < 0.01$) by dietary energy, but not by administration of rbST. Levels of serum urea were similar among treatments (Table 3). Serum Ca, P, Mg, Cu, and Zn concentrations were not affected by rbST or dietary treatment (Table 3).

Neither diet nor rbST alone had any appreciable effect on several semen measurements and the scrotal circumference determined at the end of the feeding period. Furthermore, no interaction response ($P > 0.15$) was detected; consequently, only the main effects are presented in Table 4.

Table 2. Effects of plane of nutrition and rbST administration during a 99-day feeding trial on average daily gain, height at withers and scrotal circumference in young Granadina goat bucks. Values are means ± standard error.

Variables	Nutrients[a]		rbST[b]	
	1.0	1.25	0 mg	125 mg
Initial body weight (kg)	14.7 ± 2.5	13.4 ± 4.9	14.5 ± 3.9	13.6 ± 3.8
Final body weight (kg)	25.7 ± 3.4	26.6 ± 5.9	25.8 ± 4.8	26.5 ± 4.8
ADG (g day^{-1})[cd]	111 ± 23	133 ± 25	114 ± 23	130 ± 28
Initial height at withers (cm)	50.6 ± 2.3	45.9 ± 2.4	47.6 ± 2.9	44.8 ± 3.2
Final height at withers (cm)	61.6 ± 2.6	59.2 ± 2.0	58.6 ± 3.6	57.8 ± 3.9
Change height at withers (cm)	11.0 ± 3.2	13.3 ± 3.4	11.0 ± 3	13.0 ± 3
Initial scrotal circumference (cm)	16.7 ± 2.9	14.9 ± 2.1	15.4 ± 2.0	16.4 ± 1.7
Final scrotal circumference (cm)	24.6 ± 3.2	23.8 ± 1.2	23.6 ± 2.9	25.0 ± 1.9
Change in scrotal circumference (cm)	7.9 ± 2.21	8.9 ± 2.74	8.2 ± 2.72	8.6 ± 2.34

[a]Rates of 1.0 (standard diet) and 1.25 (increased diet) times the growth energy and protein requirements.
[b]0 and 125 mg rbST applied subcutaneously every 14 days.
[c]Effect of dietary energy ($P < 0.01$).
[d]Effect of rbST ($P < 0.05$).

Table 3. Mean plasma concentration of various serum metabolites and minerals in young Granadina goat bucks fed varying dietary energy and protein levels and rbST administration during a 99-day feeding trial. Values are means ± standard error.

Variables	Nutrients[a]		rbST[b]	
	1.0	1.25	0 mg	125 mg
Glucose (mg dL^{-1})	37.5 ± 3.7	39.7 ± 5.8	38.1 ± 3.2	39.1 ± 6.3
Urea (mg dL^{-1})	19.7 ± 4.7	18.2 ± 0.8	17.8 ± 1.0	20.1 ± 4.1
Creatinine (mg dL^{-1})	0.84 ± 0.35	0.98 ± 0.27	0.90 ± 0.32	0.94 ± 0.32
Cholesterol (mg dL^{-1})[c]	80.5 ± 12.1	96.3 ± 14.5	90.8 ± 15.5	86.0 ± 15.6
Total proteins (mg dL^{-1})	9.5 ± 0.6	9.5 ± 1.0	9.6 ± 0.7	9.4 ± 0.9
Phosphorus (mg dL^{-1})	7.2 ± 0.4	7.1 ± 0.3	7.2 ± 0.4	7.1 ± 0.3
Calcium (mg dL^{-1})	10.8 ± 1.8	9.5 ± 1.3	9.8 ± 1.6	10.5 ± 1.7
Mg (mg dL^{-1})	1.9 ± 0.3	1.8 ± 0.2	1.8 ± 0.1	2.0 ± 0.3
Cu (mg dL^{-1})	1.6 ± 0.7	1.5 ± 0.5	1.7 ± 0.6	1.3 ± 0.5
Zn (mg dL^{-1})	1.2 ± 0.6	1.3 ± 0.4	1.2 ± 0.5	1.3 ± 0.6

[a]Rates of 1.0 (standard diet) and 1.25 (increased diet) times the growth energy and protein requirements.
[b]0 and 125 mg rbST applied subcutaneously every 14 days.
[c]Effect of dietary energy ($P < 0.01$).

Table 4. Effect of dietary energy and rbST administration during a 99-day feeding trial on semen production and quality of young Granadina bucks. Values are means ± standard error.

Variables[c]	Nutrients[a]		rbST[b]	
	1.0	1.25	0 mg	125 mg
Volume, mL	0.56 ± 0.32	0.48 ± 0.23	0.51 ± 0.29	0.55 ± 0.28
Sperm motility, %	63.5 ± 18.9	65.9 ± 14.9	67.1 ± 14.5	60.9 ± 19.3
Concentration ($\times 10^6$/mL)	2282 ± 1137	1946 ± 529	2210 ± 1139	2055 ± 656
Live sperm, %	69.8 ± 14.2	68.7 ± 14.9	70.4 ± 12.9	68.2 ± 16.0
Abnormal sperm, %	6.5 ± 3.4	7.8 ± 4.4	7.8 ± 3.3	6.3 ± 4.2
Total sperm ($\times 10^6$/mL)	1290 ± 926	958 ± 568	1266 ± 962	1014 ± 572

[a]Rates of 1.0 (standard diet) and 1.25 (increased diet) times thegrowth energy and protein requirements.
[b]0 and 125 mg rbST applied subcutaneously every 14 days.
[c]For all variables no statistical differences due to dietary or hormonal treatments were detected.

Discussion

Performance responses

The stimulatory effects of rbST administration on growth rate were observed throughout the experiment. This suggests that the administration of exogenous rbST to intact goat bucks, from weaning through to sexual maturity, appears to be equally effective in promoting growth rate. In accordance with the increased ADG with rbST administration, other authors (15) observed an increase in ADG in rbST-treated Angora goats.

The observation that rbST administration increased growth performance 14% in the current study is interesting, in view of the fact that the majority of the published growth trials in steers (16) and sheep (17), treated with bST during the growing and finishing phases, indicated that these animals responded with an average improvement <10% for ADG. Magnitude and efficacy of the response to bovine somatotropin in beef cattle is variable. It has been identified that the highest response of beef cattle to rbST is shortly before puberty (250 days of age; 18), a growing period similar to that of goat bucks in the present study.

The lack of increase in height at withers of rbST-treated goat bucks indicates no enhancement of long-bone growth, which contrasts with previous observations in feedlot steers (16) and ewe lambs (19) receiving rbST. No ossified epiphyseal plates were present on the metacarpal bones of 271- or 361-day-old lambs (20). Therefore, rbST should have been able to stimulate long-bone growth throughout the study, but this was not the case. Similar results to those in the present study have been reported in intact lambs (17), where no effect of GH treatment has been found in bone lengthening, growth index, and cortical index.

Serum metabolites and minerals

The lack of effect of rbST on serum glucose is consistent with the findings of previous studies on Angora (15) goats subjected to chronic administration of rbST, where glucose levels did not change, or just a transient increase of this metabolite was observed (21). The similitude in serum glucose levels between rbST-treated bucks and the control is probably due to an adjustment to a new set point in maintenance of glucose homeostasis, because of effects of GH on tissue metabolism of carbohydrates.

Blood cholesterol concentration is associated with body condition score in goats (22) and cholesterol concentration tends to increase with an increased level of nutrition during growth (23); thus, it appears that higher body energy reserves reached by bucks fed the high energy diet were reflected in higher serum cholesterol levels.

The effect of exogenous GH on carcass fat appears to be through decreased lipogenic activity in adipose tissue, wherein GH acts as an insulin antagonist (24). Likewise, prolonged treatment with GH does not affect basal lipolysis in lactating goats (25), which do not modify concentrations of cholesterol in muscle and subcutaneous fat. This seems to explain the lack of effect of rbST on serum cholesterol.

Regarding similar levels of serum urea between treatments, somatotropin increases the retention of nitrogen in beef heifers (26), apparently by enhancing the incorporation of N into muscle and proteins in noncarcass tissue (27). This increase in N retention has been reflected in a decrease in circulating concentrations of urea nitrogen (26,28). Our results are inconsistent with those previous findings, but are in line with other studies in goats (15), where the administration of rbST did not affect blood urea. Furthermore, we found no difference in the plasma total protein levels in rbST-treated and the control goats bucks, as also reported by other authors (29), which is indicative that rbST did not cause an energy deficit.

Minerals remained within the normal reference ranges for healthy goats (30), and the lack of effect of rbST on major minerals agrees with data of other authors in dairy cattle (31) and supports the concept that somatotropin coordinates mineral partitioning.

Semen traits

Over-feeding is detrimental to semen quality in young rams (32). High levels of energy intake could affect reproductive performance of young livestock used for natural mating by a direct effect on rate of sexual development (33), or by an indirect effect through degree of fatness or weight change (34). In the present study, apparently, nutrient allowances were not large enough to interfere with normal testicular function, as has been observed by other authors (35).

Taken together, these observations suggest that chronic administration of rbST does not affect the spermatogenesis process in developing goat bucks, and, consequently, sperm output. The results of the present study are in agreement with studies in stallions (36) and young bulls (37), where chronic applications of somatotropin did not alter semen quality variables.

The lack of effects of rbST on semen characteristics was possibly due to the fact that chronic treatment with rbST does not alter the pattern of LH release (38), and plasma testosterone concentration (39), which apparently did not modify testis size and gamete production. Moreover, there is evidence that doses of exogenous somatotropin similar to those used to increase milk production and carcass composition do not affect spermatogenesis in rams or bulls (40). Therefore, we reject the hypothesis that rbST or the metabolic changes resulting from its chronic administration affect the mechanisms controlling testis development and sperm cell production in goat bucks.

However, there is also evidence that somatotropin plays a role in the reproductive processes of male farm animals (41,42), although the beneficial effect of GH treatment on testicular function has been focused basically on individuals with testicular dysfunction (43).

Conclusions

High energy diets markedly increased weight gains in growing Granadina goat bucks, with and without somatotropin treatment, which implies that rapid growth rates were compatible with semen production and quality. On the other hand, these results do not depict a potential for enhancing semen production and quality through chronic rbST treatment of growing Granadina goat bucks. Further studies are still necessary to establish the effect of rbST on more subtle aspects of sperm integrity.

References

1. Mellado, M., Cárdenas, C., Ruíz, F.: Mating behavior of bucks and does in goats operations under range conditions. Appl. Anim. Behav. Sci., 2000; 67: 89-96.
2. Mellado, M., Cantú, L., Suárez, J.E.: Effect of body condition, length of the breeding period, buck:doe ratio and month of breeding on kidding rates in goats under extensive conditions. Small Rumin. Res., 1996; 23: 29-35.
3. Zarazaga, L.A., Guzmán, J.L., Domínguez, C., Pérez, M.C., R.: Effects of season and feeding level on reproductive activity and semen quality in Payoya buck goats. Theriogenology, 2009; 71: 1316-1325.
4. Almeida, A.M., Schwalbach, L.M.J., Cardoso, L.A., Greyling, J.P.C.: Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. Small Rumin. Res., 2007; 73: 216-220.
5. Mekasha, Y., Tegegne, A., Rodriguez-Martinez, H.: Feed intake and sperm morphology in Ogaden bucks supplemented with either agro-industrial by-products or khat (*Catha edulis*) leftover. Reprod. Dom. Anim., 2008; 43: 4437-4444.
6. Velayudhan, B.T., Govoni, K.E., Hoagland, T.A., Zinn, S.A.: Growth rate and changes of the somatotropic axis in beef cattle administered exogenous bovine somatotropin beginning at two hundred, two hundred fifty, and three hundred days of age. J. Anim. Sci., 2007; 85: 2866-2872.
7. Roser, J.F.: Regulation of testicular function in the stallion: an intricate network of endocrine, paracrine and autocrine systems. Anim. Reprod. Sci., 2008; 107: 179-196.
8. Closset, J., Dombrowicz, D., Vandenbroeck, M., Hennen, G.: Effects of bovine, human, and rat growth hormones on immature hypophysectomized rat testis. Growth Regul., 1991; 1: 29-37.
9. Sauerweina, H., Breier, B.H., Gallaher, B.W., Gotz, C., Kufner, G., Montag, T., Vickers, M., Schallenberger, E.: Growth hormone treatment of breeding bulls used for artificial insemination improves fertilization rates. Dom. Anim. Endocrinol., 2000; 18: 145-158.
10. Corner, E., Cassorla, F.: Growth hormone and reproductive function. Mol. Cell Endocrinol., 2002; 186: 133-136.
11. Zachman, M.. Interrelations between growth hormone and sex-hormones-physiology and therapeutic consequenses. Horm. Res., 1992; 38: 1-8.
12. Mellado, M., Meza-Herrera, C.: Influence of season and environment on fertility of goats in a hot-arid environment. J. Agric. Sci., 2002; 138: 97-101.
13. NRC.: Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids. The National Academic Press. Washington, DC, USA. 2007.
14. Fiske, C.H., Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., 1925; 66: 375-400.
15. Puchala, R., Prieto, I., Banskalieva, V., Goetsch, A.L., Lachica, M., Sahlu, T.: Effects of bovine somatotropin and thyroid hormone status on hormone levels, body weight gain, and mohair fiber growth of Angora goats. J. Anim. Sci., 2001; 79: 2913-2919.
16. Schlegel, M.L., Bergen, W.G., Schroeder, A.L., Vandehaar, M.J., Rust, S.R.: Use of bovine somatotropin for increased skeletal and lean tissue growth of Holstein steers. J. Anim. Sci., 2006; 84: 1176-1187.
17. Rosemberg, E., Thonney, M.L., Butler, W.R.: The effects of bovine growth hormone and thyroxine on growth rate and carcass measurements in lambs. J. Anim. Sci., 1989; 67: 3300-3312.

18. Govoni, K.E., Hoagland, T.A., Zinn, S.A.: The ontogeny of the somatotropic axis in Hereford calves from birth to one year of age and its response to administration of exogenous bovine somatotropin. J. Anim. Sci., 2004; 82: 1646-1655.

19. Johnsson, I.D., Hart, I.C., Butler-Hogg, B.W.: The effects of exogenous bovine growth hormone and bromocriptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. Anim. Prod., 1985; 41: 207-217.

20. Ho, L., Field, R.A., Russell, W.C., Riley, M.L., Ercanbrack, S.K., Williams Jr, F.L.: Influence of gender, breed and age on maturity characteristics of sheep. J. Anim. Sci., 1989; 67: 2460-2470.

21. Faulkner, A.: Changes in plasma and milk concentrations of glucose and IGF-1 in response to exogenous growth hormone in lactating goats. J. Dairy Res., 1999; 66: 207-214.

22. Cabiddu, A., Branca, A., Decandia, M., Pes, A., Santucci, P. M., Masoero, F., Calamari, L.: Relationship between body condition score, metabolic profile, milk yield and milk composition in goats browsing a Mediterranean shrubland. Livest. Prod. Sci., 1999; 61: 267-263.

23. Wehrman, M.E., Welsh Jr, T.H., Williams, G.L.: Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of postpartum luteal activity. Biol. Reprod., 1991; 45: 514-522.

24. Vernon, R.G.: Effects of growth hormone on fatty acid synthesis in sheep adipose tissue. Int. J. Biochem., 1982; 14: 255-258.

25. Vernon, R.G., Faulkner, A., Finley, E., Watt, P.W., Zammit, V.A.: Effects of prolonged treatment of lactating goats with bovine somatotropin on aspects of adipose tissue and liver metabolism. J. Dairy Res., 1995; 62: 237-248.

26. Eiseman, J.H., Hammond, A.C., Bauman, D.E., Reynolds, P.J., McCutcheon, S.N., Tyrrell, H.F., Haaland, G.L.: Effect of bovine growth hormone administration on metabolism of growing Hereford heifers: Dietary digestibility, energy and nitrogen balance. J. Nutr., 1986; 116: 157-163.

27. Early, R.J., McBride, B.W., Ball, R.O.: Growth and metabolism in somatotropin-treated steers: I. Growth, serum chemistry and carcass weights. J. Anim. Sci., 1990; 68: 4134-4143.

28. McShane, T.M., Schillo, K.K., Estienne, M.J., Boling, J.A., Bradley, N.W., Hall, J.B.: Effects of recombinant DNA derived somatotropin and dietary energy intake on development of beef heifers: II. Concentrations of hormones and metabolites in blood sera. J. Anim. Sci., 1989; 67: 2237-2244.

29. Sallam, S.M.A., Nasser, M.E.A., Yousef, M.I.: Effect of recombinant bovine somatotropin on sheep milk production, composition and some hemato-biochemical components. Small Rumin. Res., 2005; 56: 165-171.

30. McDowell, L.R. 1985. Nutrition of grazing ruminants in warm climates. Academic Press Inc., New York.

31. Law, F.M., Leaver, D.D., Martin, T.J., Selleck, K., Clarke, I.J., Moate, P.J.: Effect of treatment of dairy cows with slow-release bovine somatotropin during the periparturient period on minerals in plasma and milk and on parathyroid hormone-related protein in milk. J. Dairy Sci., 1994; 77: 2242-2248.

32. Fourie, P.J., Schwalbach, L.M., Neser, F.W.C., Van Der Westhuizen, C.: Scrotal, testicular and semen characteristics of young Dorper rams managed under intensive and extensive conditions. Small Rumin. Res., 2004; 54: 53-59.

33. Nolan, C.J., Neuendorff, D.A., Godfrey, R.W., Harms, P.G., Welsh Jr., T.H., Mcarthur, N.H., Randel, R.D.: Influence of dietary energy intake on prepubertal development of Brahman bulls. J. Anim. Sci., 1990; 68: 1087-1096.

34. Almquist, J.O.: Effect of long-term ejaculation at high frequency on output of sperm, sexual behaviour, and fertility of Holstein bulls; relation of reproductive capacity to high nutrient allowance. J. Dairy Sci., 1982; 65: 814-823.

35. Pruitt, R.J., Corah, L.R.: Effect of energy intake after weaning on the sexual development of beef bulls. I. Semen characteristics and serving capacity. J. Anim. Sci., 1985; 61: 1186-1193.

36. De Botton, D., Janett, F., Burger, D., Imboden, I., Kähn, W., Thun, R.: Influence of long-term treatment with equine somatotropin (EquiGen) on gonadal function in stallions with poor semen quality. Schweizer Archiv Tierheilkunde, 2008; 150: 157-165.

37. MacDonald, R.D., Deaves, D.R.: Testicular development in bulls treated with recombinant bovine somatotropin. J. Anim. Sci., 1993; 71: 1540-1545.

38. Hall, J.B., Schillo, K.K., Fitzgerald, B.P., Bradley, N.W.: Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development, and onset of puberty in beef heifers. J. Anim. Sci., 1994; 72: 709-718.

39. Storer, W., Thompson, J.R.D., Cartmill, J.: The effects of equine somatotropin on pituitary and testicular function in the stallion during the nonbreeding season. J. Equine Vet. Sci., 2005; 25: 106-112.

40. N'Deaver, D.R., Bryan, K.A.: Effects of exogenous somatotropin (ST) on gonadal function in ruminants and swine. Dom. Anim. Endocrinol., 1999; 17: 287-297.

41. Vieira, M., Bianchi, I., Madeira, E., Roll, V., Oliveira, C., Viau, P., Pivato, I., Severo, N., Del Pino, F., Schneider, A., Corrêa, M.: Effect of recombinant bovine somatotropin on plasma concentrations of insulin-like growth factor I, insulin and membrane integrity of bull spermatozoa. Reprod. Dom. Anim., 2010; 45: 1110–1113.

42. Aurich, J. E., Kranski, S., Parvizi, N., Aurich, C.: Somatostatin treatment effects testicular function in stallions. Theriogenology, 2003; 60: 163-174.

43. Ovesen, P.G., Jørgensen, J.O., Ingerslev, J., Orskov, H., Christiansen, J.S.: Growth hormone treatment of men with reduced sperm quality. Ugeskrift Laeger, 1998; 160: 176-180.