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Effect of two dietary protein levels on testosterone, testicular parameters and semen quality in ram lambs during pubertal development

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Summary

The aim of this study was to determine the effects of two dietary protein levels on testosterone, testicular parameters and semen quality in Kivircik ram lambs during pubertal development. Two experimental groups were formed. Following weaning, crude protein (CP) and metabolic energy (ME) levels were 12% CP, 2.54 Mcal/kg in group I (low protein diet) and 18% CP, 2.52 Mcal/kg in group II (high protein diet). Measurements of live weight and testicular characteristics and collection of blood samples for testosterone hormone concentrations were performed at 20 day intervals starting from 115-days- up to 195-days-of-age. There was an increase in semen volume, spermatozoa concentration and the percentage of progressively motile sperm in both groups between 135 and 195 days of age. Group I had significantly higher semen volume on day 175 ($P < 0.05$). Furthermore, spermatozoa concentrations were higher in group I than those in group II on days 155 and 175 ($P < 0.05$). Values of live weight, testicular diameter, testicular circumference, testicular length and testicular volume of ram lambs in group II (high protein diet) were higher than those in group I (low protein diet). Testicular length and testicular volume of group II were significantly higher than those of group I on day 195 ($P < 0.05$). Despite the fact that group II had an alternating testosterone hormone concentration, it was determined that Group II had better testosterone hormone concentration values than group I on day 115, 135 and 175. However, group I had a higher testosterone hormone level on day 155 and 195. Live weight and testicular characteristics of ram lambs fed with a high protein diet were affected positively during pubertal development. However, it was observed that feeding with a high protein diet had a negative effect on semen characteristics by impairing the thermoregulation mechanism and spermatogenesis in testicles because of excessive fat accumulation in the scrotum.

Keywords: ram lambs, reproduction traits, feed, protein levels

Nutrition plays a major role in many aspects of male reproduction, including the attainment of sexual maturity, both in terms of spermatogenesis and libido (5). The study of the reproductive function in different ruminant species has provided evidence for the effects of nutrition during the growing period on the development of the hypothalamic-pituitary gonadal axis and hence on the onset of puberty. It has been reported that low levels of nutrition during the prepuberal period in ruminants delay testicular growth and the onset of puberty by inhibiting the development of a mature reproductive endocrine system (17). There is now considerable evidence suggesting that the influence of nutrition on the reproductive processes is mediated via effects of dietary constituents on the hypothalamic-pituitary axis, although there is some indication that dietary changes may affect the testis directly (4). Although undernutrition negatively influences the attainment of puberty (8), the mechanism by which nutrition influences reproduction is largely unknown. However, it is noteworthy that there is some controversy about the effects of additional nutritive supply above maintenance requirements during the prepubertal period on testicular development and semen characteristics. It has been reported that the reproductive potential of young males may also be impaired by overfeeding. Coulter and Kozub (6) observed a detrimental effect of high energy intake on 2-year-old Hereford bulls,

as measured by epididymal sperm reserves and sperm motility and morphology. Morrow et al. (14) evaluated the effects of low and high energy diets on the growth and reproductive development of Angus and Simmental bulls and found that the low energy group showed higher values of fertility and libido. In contrast to the above-mentioned results, several studies did not reveal any effect of the level of nutrient intake during the pre and postpubertal period of young bulls and rams on reproductive traits, such as testicular size, semen quality or serving capacity, or the attainment of puberty (3, 17). It has been reported that reproductive characteristics of ram lambs were effected by different feeding in early stages of their life, and these differences were compensated by the improvement of feeding (19).

This study was carried out in order to determine the effects of feeding Kivircik ram lambs during the pubertal period with two diets that have the same energy levels but different protein levels on growth performance, testicle morphology, testosterone hormone concentration and semen quality.

Material and methods

This research was conducted in the boxes of Education and Research Hospital in the Veterinary Faculty, University of Istanbul. Twenty single-born ram lambs were used for the research. Ram lambs were housed together with the other lambs starting from lambing until weaning. The lambs were weaned at three--months-of-age. During the suckling period, lambs were fed with lucerne and concentrate feed. Following weaning, lambs were transported to experimental pens. Ram lambs were randomly assigned into two groups (n = 10). The experimental groups were designed according to the percentage of crude protein and source of protein of the diet. In addition, the energy levels of the diets in both groups were kept equal. Crude protein (CP) and metabolic energy (ME) levels were 12% CP, 2.54 Mcal/kg in group I (low protein diet) and 18% CP, 2.52 Mcal/kg in group II (high protein diet) (tab. 1). For fiber intake, only high quality lucerne was fed. At the beginning of the trial lambs were fed with 600 g/head/day concentrate feed and 200 g/head/day lucerne on average. During the course of the experiment amounts of concentrate feed and lucerne were increased up to 1000 g/head/day and 400 g/head/day. Drinking water was available continuously during the experiment period. Data was collected for the first time when the ram lambs were 115-days--old. Measurements of live weight and testicular characteristics (testicular diameter, testicular length, scrotal circumference and testis volume), and collection of blood samples for plasma testosterone concentrations were taken every 20 days until the end of the experiment. Sperm was evaluated for each 20-day interval starting from 135 until 195-days-of-age. Live weight was recorded in the morning before feeding. Testicular diameter was recorded with a caliper on the left and right testicles as the widest anteroposterior diameter. Testicular length was also measured with a caliper both on the left and right testicles as the distance between the top of the tail and the head of the epididymis. Scrotal circumference was measured with a flexible tape at the point of maximum circumference of paired testes. Paired testicular volume were calculated by $0.0396 \times (\text{average testis length}) \times (\text{scrotal circumference})^2$ (10).

Semen was collected from rams using a manually controlled electro-ejaculator (P-T Electronics, Model 304, USA) with a rectal probe that has three electrodes. The rectal probe was lubricated and gently inserted into the rectum, and orientated so that the electrodes were positioned ventrally. Electric current was applied starting from 1 volt for 2 sec with 2-sec rest intervals between stimuli, increasing the voltage stimuli by one volt at a time. The penis was prolapsed beyond the prepuce, and semen was collected into a graduated collection vial attached to an artificial vagina at room temperature. Collected semen were immediately transported to the laboratory and immersed in a water bath at 30°C. The volume of the ejaculates was read directly from a graduated collection container with 0.1 ml intervals. The spermatozoa concentration was determined by optical density with a spectrophotometer (Photometer SDM4, Minitüb, Germany) calibrated for ram species (1 : 1000 dilution rate). A small subsample of semen was diluted with physiological saline on a slide, covered with a cover slip and placed on a microscope stage at 37°C. The percentage of progressively motile sperm was estimated qualitatively by examining approximately eight fields at a magnification of 400 × (11). To avoid variance, all semen measurements were analyzed by a single researcher in this study. Blood samples from each ram lambs were collected by jugular venipuncture, every 20 days starting from 115 to 195-days-of-age. Collection of blood samples was completed in one hour. After collection, blood samples were centrifuged at 1200 × g for 15 minutes, and serum samples were stored at –20°C until assayed. Serum testosterone concentrations were measured in a double antibody radioimmunoassay (RIA) using a testosterone RIA Kit (DSL-4000 ACTIVE® Testosterone Coated-Tube Radioimmunoassay Kit, Diagnostic Systems Laboratories, Inc., USA). The procedure follows the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [1-125]-labeled testosterone is present. The separation of the free and bound antigen is achieved by decanting or aspirating the antibody-coated tubes. For measurement of serum testosterone concentrations, 6 standards, 0, 0.1, 0.5, 2.5, 10.0 and 25.0 ng/ml, and 2 control kits were used. The sensitivity of this method was 0.08 ng/ml and intra-and inter--assay coefficients of variation were 7.8 and 8.4%, respectively.

In the statistical analysis, all the related characteristics were investigated (live weight, testicular characteristics, testosterone concentrations and semen characteristics). Independent Samples t-test was used to observe whether any differences existed between groups (21). Calculations were made using the SPSS program pack (16).

Tab. 1. Formulation and chemical composition of the experimental diets

Ingredients	Group I (Low protein diet)	Group II (High protein diet)
Lucerne	15	15
Wheat bran	10	13
Barley grain	69.5	47.5
Soybean meal	2	21
Salt	1	1
Sodium bicarbonate	0.5	0.5
Limestone	1.5	1.5
Vitamins and mineral mix*	0.5	0.5
Total	100	100
Calculated chemical composition (% DM basis)		
Dry matter (%)	90.09	90.06
Crude protein (%)	12	18
ME (Mcal/kg)	2.54	2.52

Results and discussion

The effects of nutrition on live weight, testicular characteristics, testosterone hormone level and semen quality during the pubertal development period of the ram lambs have been investigated in this study. The results for the semen characteristics are presented in tab. 2. There were increases in semen quality values in both groups between 135 and 195-days-of-age. Although increases in these values were generally higher in group II (low protein diet), there were statistical differences ($P < 0.05$) for semen volume only on day 175, and for spermatozoa concentration on days 155 and 175. No statistical difference was observed between the groups in terms of motile spermatozoa during the study. The measurements on the 195th day of the study coincided with the quality season. No difference between the groups in terms of semen quality was observed for this period. Abi Saab et al. (1) have reported different results than the findings of this study, according to which the semen volume and the spermatozoa concentrations of the group that was fed with a high protein diet (18%) during the pubertal period were better than the group fed with a low protein diet (12%) as a result of investigations they conducted on 16 Baladi male goats.

The developments of live weight and testicular characteristics are presented in tab. 3. Values for live weight, testicular diameter, testicular circumference, testicular length and testicular volume of rams in group I were higher compared to those in group II for all observations (on days 115, 135, 155, 175 and 195). An increase was observed in all parameters from day 115 to day 195 in both groups. While no statistically significant difference was found between two groups on days 115, 135, 155 and 175 in all parameters, there was a statistical difference on day 195 for testicular length and volume between the two groups ($P < 0.05$). The live weight and testicular parameters of the ram lambs in group II (high protein diet) were higher than the ram lambs in group I (low protein diet) for all the time intervals included in the study (at days 115, 135, 155, 175 and 195). No statistical difference has been observed between the groups in terms of the above mentioned parameters with the exception of the differences of testicular length and testes volume on the 195th day between the two groups that were statistically different ($P < 0.05$). Rekwot et al. (18) have also reported similar results indicating that the live weight and properties of the scrotum area of the young bulls that were fed with two different diets which had equal energy levels but different protein levels were higher in the high protein diet group. The study of Abi Saab et al. (1) supports the results of our study; they reported that 16 Baladi male goats fed with a high protein feed (18%) reached puberty at 22 weeks and 23.8 kg live weight; whereas those goats fed with a low protein feed (12%) reached puberty at 31-weeks-of-age and 26.7 kg live weight. The findings are parallel to those of Mukasa-Mugerva and Ezaz (15) and Foruie et al. (9). Besides the results of the studies mentioned above, the results of live weight and testicular parameters obtained from this study are different than the results other researchers have reported. Some researchers have reported that the optimum protein level that must be in the diet during the growth period of the ram lambs should decrease to 12% from 18% as the live weight increases (2, 13).

Tab. 2. Mean (± S.E.) of semen characteristics (semen volume, spermatozoa concentration and motile spermatozoa) in ram lambs from 135 to 195 days

Days	Groups	n	Semen volume (ml)	Spermatozoa concentration (..× 10^9/ml)	Motile spermatozoa (%)
135	I	5	0.48 ± 0.10	0.56 ± 0.26	23.0 ± 15.3
	II	6	0.48 ± 0.12	0.27 ± 0.14	14.2 ± 12.3
155	I	10	0.99 ± 0.07	0.97 ± 0.10^a	63.5 ± 2.9
	II	10	0.75 ± 0.08	0.51 ± 0.11^b	60.5 ± 4.6
175	I	9	1.12 ± 0.13^a	1.00 ± 0.14^a	70.0 ± 4.1
	II	10	0.88 ± 0.15^b	0.58 ± 0.14^b	65.0 ± 6.4
195	I	10	1.14 ± 0.11	1.42 ± 0.21	72.0 ± 3.1
	II	10	0.92 ± 0.06	1.40 ± 0.25	69.0 ± 4.2

Explanations: a, b – Means within a row with different superscripts are significantly different ($P < 0.05$)

Tab. 3. Mean (± S.E.) value of live weight and testicular characteristics at different times throughout the experiment for ram lambs

Days	Groups	n	Live weight (kg)	Scrotal circumference (cm)	Testicular diameter (cm)	Testicular length (cm)	Testes volume (cm^3)	Testosterone (ng/ml)
115	I	10	27.3 ± 1.2	18.6 ± 0.9	2.98 ± 0.2	6.28 ± 0.5	93 ± 17	1.00 ± 0.3
	II	10	27.8 ± 0.9	18.7 ± 0.9	2.93 ± 0.2	6.76 ± 0.4	100 ± 16	1.24 ± 0.3
135		10	28.9 ± 1.1	21.1 ± 1.3	3.69 ± 0.3	7.95 ± 0.4	152 ± 23	1.26 ± 0.6
	II	10	30.6 ± 1.2	21.8 ± 1.2	3.79 ± 0.2	8.13 ± 0.4	164 ± 25	2.05 ± 0.5
155	I	10	31.4 ± 1.3	24.8 ± 1.3	4.37 ± 0.3	9.12 ± 0.4	233 ± 27	1.46 ± 0.6
	II	10	35.2 ± 1.7	27.7 ± 0.9	4.77 ± 0.2	9.90 ± 0.4	310 ± 34	0.75 ± 0.2
175	I	10	36.1 ± 1.5	26.8 ± 1.3	4.56 ± 0.3	9.47 ± 0.4	282 ± 33	0.92 ± 0.2
	II	10	39.8 ± 1.4	28.9 ± 0.9	5.10 ± 0.2	10.4 ± 0.4	354 ± 33	1.82 ± 0.6
195		10	39.3 ± 1.5	28.3 ± 0.8	5.06 ± 0.2	10.7 ± 0.4^b	344 ± 27^b	2.09 ± 0.4^a
	II	10	42.8 ± 1.2	30.5 ± 0.9	5.24 ± 0.2	11.7 ± 0.3^a	437 ± 30^a	0.82 ± 0.2^b

Explanations: as in tab. 2.

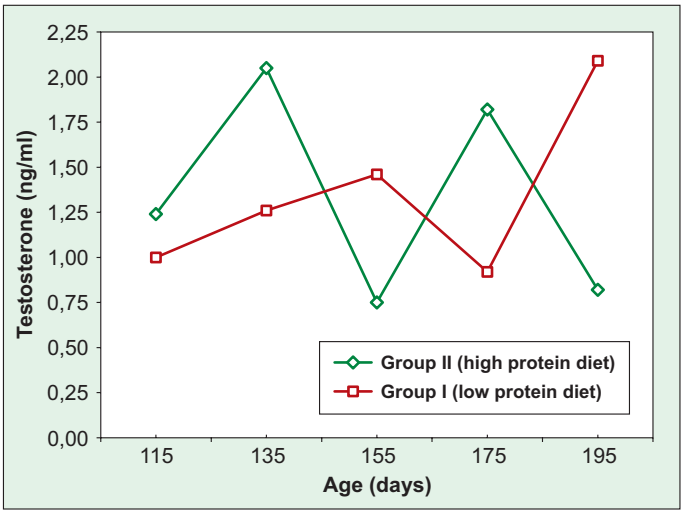

Fig. 1. Serum testosterone concentrations in Group I (low protein diet) and Group II (high protein diet) from 115 to 195 days

Rekwot et al. (18) have reported that there is no difference in the semen in terms of volume between the groups of young bulls that were fed with two different diets that had equal energy levels but different protein levels. The two studies are in harmony since both report similar results. Foruie et al. (9) reported similar results which reveal that the increase in the live weight and testicular parameters of the Dorper race male lambs, which were fed with diets that contained high levels of protein, were higher compared to the low protein group; however, more optimal results have been obtained from the low protein group in terms of the spermatological parameters compared to the high protein group. Similarly, higher motility, density and semen volume value have been obtained from group I (low protein diet) compared to group II (high protein diet) during the entire research period. It is assumed that the results that have been obtained are a because of the fact that a high protein diet results in the excess fat to be stored in the scrotum, thus the thermoregulation mechanism in the testis and the spermatogenesis to collapse.

The development of serum testosterone concentrations is presented in fig. 1. There was no significant effect of diet on circulating the concentration of this hormone at any sampling time. A steady increase has been observed at the testosterone hormone levels of the ram lambs in group I during the study period. As expected, plasma concentration of testosterone increased with chronological age. In general, the increase in the serum testosterone concentrations is linear during the pre-pubertal period and it reaches to a maximum value between the ages of 5 and 7 months. A classical theory proposed a GnRH-dependent pathway to explain the effect of protein nutrition, where changes in testicular size caused by increases and decreases in protein supply were positively correlated with changes in the secretion of gonadotrophins and testosterone (12, 20). Fluctuations were observed at the serum testosterone concentration values between the groups during the study. Fernandez et al. (7) have reported that the testosterone hormone concentration had fluctuations for the Assaf race rams which were fed with low, medium and high protein diets. Since the data obtained during this study are similar with the testosterone hormone concentration oscillation in Fernandez et al. (7) the two studies are parallel to each other.

The results show that diets with equal energy levels but different protein levels have different effects on the live weight, testicular parameters, testosterone hormone concentration and sperm parameters of the ram lambs. It has been determined that the live weight and testicular parameters of the ram lambs that were fed with high protein diets during the pubertal period have been affected positively. In conclusion, feeding with a high protein diet had a negative effect on semen characteristics on account of the collapsed thermoregulation mechanism and spermatogenesis in testicles caused by excessive fat accumulation in the scrotum. The testosterone hormone concentration had a fluctuating trend independent from the diet type. Research on the effects of diets with different energy and protein levels on the testicular and spermatogenesis parameters of the ram lambs will be beneficial and contribute to the existing literature.

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