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EFFECT OF DIFFERENT PROTEIN LEVELS ON, TESTICULAR PARAMETERS AND SEMEN QUALITY IN KIVIRCIK RAM LAMBS DURING PUBERTAL DEVELOPMENT

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ABSTRACT – Effect of Different Protein Levels on, Testicular Parameters and Semen Quality in Kivircik Ram Lambs During Pubertal Development
The aim of this study was to determine the effects of different protein levels on, testicular parameters and semen quality in Kivircik ram lambs during pubertal development. Two experimental groups were formed. Following weaning, crude protein (CP) were 12% CP in group I (low protein diet) and 18% CP in group II (high protein diet). Measurements of live weight and testicular characteristics were performed in 20 days 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 concentration 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 ($P<0.05$). Live weight and testicular characteristics of ram lambs fed with high protein diet were affected positively during pubertal development. However, it was observed that feeding with high protein diet had negative effect on semen characteristics by impaired thermoregulation mechanism and spermatogenesis in testicles because of excessive fat accumulation in scrotum.

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

INTRODUCTION

Nutrition plays a major role in many aspects of male reproduction, including attainment of sexual maturity, both in terms of spermatogenesis and libido (CARPENTER et al., 1997). 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 planes 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 (PRUITT AND CORAH, 1985).

There is now considerable evidence suggesting that the influence of nutrition on 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 (BROWN, 1994). Undernutrition negatively influences attainment of puberty (FOSTER et al., 1998). However, the mechanism by which nutrition influences reproduction

is largely unknown. Nevertheless, it is noteworthy that there is some controversy about the effects of additional nutritive supply, above maintenance requirements, during the pre-pubertal period on testicular development and semen characteristics.

Thus, it has been reported that the reproductive potential of young males may also be impaired by overfeeding. COULTER AND KOZUB (1984), 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. (1981) 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, attainment of puberty (BIELLI et al., 2001). It was reported that reproductive characteristics of ram lambs were effected by different feeding in early stage of their life, and these differences were compensated by improving of feeding (SUTAMA and EDEY, 1986).

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

MATERIAL AND METHODS

This research was conducted in the boxes of Education and Research Hospital in Veterinary Faculty, University of Istanbul.
Twenty single-born ram lambs were used for the research. Kıvırcık ram lambs were housed together with the other lambs starting from lambing until weaning. The lambs were weaned at three months 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 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 has been 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) were taken every 20 days until the end of the experiment.
Sperm was evaluated for each 20 days interval starting from 135 until 195 days of age.

Table 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

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 x (average testis length) x (scrotal circumference)^2 (GODFREY, 1998). 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 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. Volume of 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 400x (MARCO-JIME'NEZ, 2005). To avoid variance, all semen measurements were analyzed by a single researcher in this study.

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 (ZAR, 1996). Calculations have been made using the SPSS program pack (OZDAMAR, 1999).

RESULTS

The effects of nutrition on live weight, testicular characteristics 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 Table 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 I (low protein diet), there were statistical differences ($P < 0.05$) for semen volume on day only 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.

Table 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 (..x 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

$^{a, b}$ Means within a row with different superscripts are significantly different ($P < 0.05$).

The developments of live weight and testicular characteristics are presented in Table 3. Values for live weight, testicular diameter, testicular circumference, testicular length and testicular volume of rams in group I were lower 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 statistically no 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). While no statistical difference has been observed between the groups in terms of the above mentioned parameters, but only the differences at testicular length and testes volume on the 195th day between the two groups were statistically different ($P<0.05$).

Table 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)
115	I	10	27.3 ± 1.2	18.6 ± 0.9	2.98 ± 0.2	6.28 ± 0.5	93 ± 17
	II	10	27.8 ± 0.9	18.7 ± 0.9	2.93 ± 0.2	6.76 ± 0.4	100 ± 16
135	I	10	28.9 ± 1.1	21.1 ± 1.3	3.69 ± 0.3	7.95 ± 0.4	152 ± 23
	II	10	30.6 ± 1.2	21.8 ± 1.2	3.79 ± 0.2	8.13 ± 0.4	164 ± 25
155	I	10	31.4 ± 1.3	24.8 ± 1.3	4.37 ± 0.3	9.12 ± 0.4	233 ± 27
	II	10	35.2 ± 1.7	27.7 ± 0.9	4.77 ± 0.2	9.90 ± 0.4	310 ± 34
175	I	10	36.1 ± 1.5	26.8 ± 1.3	4.56 ± 0.3	9.47 ± 0.4	282 ± 33
	II	10	39.8 ± 1.4	28.9 ± 0.9	5.10 ± 0.2	10.4 ± 0.4	354 ± 33
195	I	10	39.3 ± 1.5	28.3 ± 0.8	5.06 ± 0.2	10.7 ± 0.4 [b]	344 ± 27 [b]
	II	10	42.8 ± 1.2	30.5 ± 0.9	5.24 ± 0.2	11.7 ± 0.3 [a]	437 ± 30 [a]

[a, b] Means within a row with different superscripts are significantly different ($P < 0.05$).

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.

CONCLUSIONS

The results show that diets with equal energy levels but different protein levels have different effects on the live weight, testicular parameters and sperm parameters of the Kivircik 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 high protein diet had negative effect on semen characteristics by collapsed thermoregulation mechanism and spermatogenesis in testicles because of excessive fat accumulation in scrotum. A 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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