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Effect of improved diet on semen quality and scrotal circumference in the ram

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ABSTRACT

The aim of this investigation was to assess the effect of improved diet above maintenance requirement on reproductive parameters, including testicular size, semen volume, sperm concentration and viability. Twelve Bakhtiary rams were allocated to two groups of six animals and were fed during a 12-week experiment period with different diets which were designed to supply maintenance and above maintenance requirements (dry matter, energy and protein). Dry matter (DM), barley and soybean meal for control and treatment groups were 1.5 kg, 8%, 0% and 2.1 kg, 25%, 17%, respectively. Semen was collected by artificial vagina every other week. Scrotal circumference, sperm concentration and total number of spermatozoa per ejaculate significantly increased in treatment group ($P<0.05$). However, neither semen volume nor proportion of live and dead spermatozoa was affected ($P>0.05$). Likewise, there was a significant correlation between scrotal circumference and sperm production in treatment group ($P<0.01$, $r = 0.96$). It was concluded that improved dietary intake with higher energy and protein supplementation in Bakhtiary rams can improve their reproductive performance during the breeding season.

Key words: ram, semen, scrotal circumference, diet

Introduction

Reproductive well-being and performance of farm animals is largely dependent on their nutritional status. It is well documented that adequate nutritional management is crucial for successful mating in sheep flocks (FERNANDEZ et al., 2004; SMITH and AKINBAMIJO, 2000). Several studies have documented the interrelationship between energy intake and reproductive performance in adult rams (MURRAY et al., 1990; ROWE and MURRAY, 1984;

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BRADEN et al., 1974) and there is no doubt that protein deficient feeding can reduce semen quality and sexual activity (BROWN, 1994; OKOLSKI et al., 1971).

Carbohydrate, protein and nucleic acid metabolism and their deficiency may impair spermatogenesis and libido in males and fertility, embryonic development and survival, post-partum recovery activities, milk production, offspring development and their survival in females (MITCHELL et al., 2003; ALEJANDRO et al., 2002; SMITH and AKINBAMIJO, 2000).

There are several studies concerning the relationship between nutrition and fertility in the ewe. For example, it has been proved that improved diet in the ewe increases ovulation rate and modifies the morphological and functional quality of the oocytes and embryo recovered (LOZANO et al., 2003; BOLAND et al., 2001; NOTTLE et al., 1997; ARTHUR et al., 1996). There is a number of studies concerning the effects of special nutrients, particularly proteins, (with or without day length) on reproductive parameters in the ram. Nevertheless, the effects of protein supply above maintenance requirements are more equivocal, with reports of either an increase or no changes in reproductive parameters, such as testicular size, semen quality, testosterone secretion or sexual activity (FERNANDEZ et al., 2004; BIELLI et al., 1999; BOUKHLIQ et al., 1997; MARTIN et al., 1994; LINDSAY et al., 1984; OLDHAM et al., 1978). However, there is no literature pertaining to the effect of improved diet, including increase in dry matter (DM) with higher energy and protein supplement than maintenance on semen quality, especially its volume and concentration, as important factors in semen evaluation and scrotal circumference (as a direct indicator of sperm production) in the ram.

The aim of this study was to determine whether or not improved diet could affect semen quality and testicular size in comparison with maintenance nutrition in the ram.

Materials and methods

Animals and diets. This study was conducted at the farm of Lorestan University (Latitude: 33˚ 29´N, Longitude: 48º 22´ E, Altitude: 1125 m) in Lorestan Province in Iran during the months of November 2004 to January 2005. Twelve sexually active Bakhtiary rams (the native sheep breed in Iran) aged between 11 and 14 months and weighing 52.3 ± 4.6 kg were allocated to two groups during the experiment. All animals had been reared under similar conditions at the farm. Two diets were designed and formulated according to NRC (1985) to feed either the recommended (control; n = 6) or improved diet (treatment; n = 6). The balanced diet of two groups is shown in Table 1.

Experimental procedure. The trial lasted 12 weeks during the breeding season under natural photoperiod and temperature conditions. During the experimental period the animals were housed individually in pens where fresh water was freely available. Each ram received a specific amount of roughage and concentrate calculated on the basis of live body mass, distributed in two equal meals at approximately 09:00 and 18:00 h.

Scrotal circumference (SC) was recorded by spermatic cord grasping in the first week until end of experiment in cm. The rams were trained for semen collection into an artificial vagina (AV) using two receptive restrained ewes treated with oestrogen 1 to 2 days previously (1 mg estradiol benzoate, Aburaihan Pharmaceutical Co., Iran). Semen of two groups of rams was collected every other week using AV, which is the most reliable method for laboratory sperm evaluation. During the first 3 weeks of trials semen collection was achieved several times in order to assess epididimal depletion of previously produced and reserved sperm.

The ejaculates were assessed for volume, sperm concentration, total number of spermatozoa per ejaculate (volume × sperm concentration) and the proportion of live and dead spermatozoa. Sperm concentration was measured using Neubauer haemocytometer counting chamber according to the method of the World Health Organization (1992). For determination of sperm viability percentage, sperms stained with dual stain technique using eosin 1% and aniline blue 4%. were analysed

Statistical analysis. Results were analyzed using SPSS version 10. In order to make a comparison of the SC, semen volume, sperm concentration, total number of spermatozoa and viability between groups, upon evaluation of equality of variances, Levene's test, independent sample t-test was applied. The correlation between scrotal circumference and sperm concentration was measured by Pearson correlation test (PETRIE and WATSON, 1999). Data was reported as mean ± S.E.M. Values were considered to be statistically significant at $P<0.05$.

Results

All results have been summarized in Table 2.

Scrotal circumference. There was a significant difference between groups in SC from week 5 to the end of the experiment ($P<0.05$). The changes before this time were not significant. The mean diameter of SC in the control and treatment groups in weeks 1 and 12 were 27.1 ± 0.09, 29.4 ± 0.16 and 26.9 ± 0.17, 30.8 ± 0.10, respectively.

Semen volume. As shown in Table 2, there was a tendency for semen volume to increase ($P = 0.073$) in week 7, so that at this time the mean of semen volume reached to 0.88 ± 0.04 mL in treatment group, in comparison with 0.76 ± 0.03 mL in the control group. However, this difference was not statistically significant between groups throughout the experiment.

Sperm concentration and total number of spermatozoa. There was a significant increase in sperm concentration in week 7 from the beginning of the trial to the end of the experiment ($P<0.05$). Likewise, total number of spermatozoa per ejaculate was significantly increased ($P<0.05$). Also, there was a high relationship between SC and sperm production

in the treatment group (P<0.01). The correlation coefficient between these parameters was r = 0.96.

Sperm viability. There was no significant difference between groups in the proportion of live spermatozoa throughout the experiment.

Table 1. Compositions of balanced diet for each ram in two experimental groups

	Control	Treatment
DM (gr)	1500	2100
Alfalfa (%BW) (DM = 90%, M.E. = 0.63 Mcal/kg, C.P. = 16%)	60	58
Barley (%BW) (DM = 88.89%, M.E. = 2.54 Mcal/kg, C.P. = 11.9%)	8	25
Soybean meal (%BW) (DM = 89.1%, M.E. = 2.31 Mcal/kg, C.P. = 43.6%)	-	17
Wheat straw (%BW) (DM = 90.8%, M.E. = 0.49 Mcal/kg, C.P. = 2.7%)	32	-

Table 2. Mean (± SEM) values of reproductive traits at different times throughout the experiment in two groups of rams

	1	3	5	7	9	11	12
SC (cm)							
Control	27.1 ± 0.09	27.4 ± 0.2	27.7 ± 0.20^{a}	28 ± 0.18^{a}	28.5 ± 0.18^{a}	29 ± 0.18^{a}	29.4 ± 0.16^{a}
Treatment	26.9 ± 0.17	27.4 ± 0.19	28.3 ± 0.10^{b}	28.9 ± 0.08^{b}	29.6 ± 0.17^{b}	30.4 ± 0.18^{b}	30.8 ± 0.10^{b}
Semen volume (mL)							
Control	0.81 ± 0.03	0.8 ± 0.02	0.83 ± 0.04	0.76 ± 0.03	0.81 ± 0.03	0.85 ± 0.04	0.88 ± 0.04
Treatment	0.78 ± 0.04	0.81 ± 0.04	0.88 ± 0.04	0.88 ± 0.04	0.90 ± 0.05	0.93 ± 0.04	0.90 ± 0.03
Concentration ($\times 10^{9}$)							
Control	4.11 ± 0.05	4.02 ± 0.05	4.16 ± 0.05	4.07 ± 0.08^{a}	4 ± 0.05^{a}	3.97 ± 0.05^{a}	3.98 ± 0.06^{a}
Treatment	4.14 ± 0.07	4.07 ± 0.1	4.22 ± 0.08	4.32 ± 0.06^{b}	4.48 ± 0.04^{b}	4.52 ± 0.04^{b}	4.55 ± 0.04^{b}
Sperm Viability (%)							
Control	87 ± 1.2	85.5 ± 1.4	84.5 ± 1.2	86.3 ± 1.4	86.8 ± 0.9	85.8 ± 1.9	87 ± 0.7
Treatment	87.1 ± 1.3	86.6 ± 0.8	85.6 ± 0.9	86.5 ± 0.9	87.5 ± 0.7	86.5 ± 0.7	86.8 ± 1.1

Means with different superscripts (a, b) within each column are significantly different (P<0.05).

Discussion

Reproductive performance of livestock is determined by four factors: genetic merit, physical environment, nutrition and management. Evidence from the literature and practical experience suggests that nutritional factors are perhaps the most crucial in terms of their direct effects on reproductive phenomenon and the potential to moderate the effects of other factors. Thus, adequate nutrition could encourage mediocre biological types to reach their genetic potential, alleviate the negative effects of a harsh physical environment and minimize the effects of poor management techniques. Moreover, nutritional factors, more than any others, readily lend themselves to manipulations to ensure positive outcomes (SMITH and AKINBAMIJO, 2000).

As indicated by SC, testicular size was affected by nutrition, with SC value being lower for control than improved diet. This result gives support to the hypothesis that testicular growth can be affected when animals are fed above their maintenance requirement. Our result is in agreement with those obtained by FERNANDEZ et al. (2004), HOTZEL et al. (2003), BOUKHLIQ et al. (1997), OLDHAM et al. (1978), although inconsistent with those obtained by BIELLI et al. (1999) and LINDSAY et al. (1984), who found no significant effect from improved pasture or high dietary protein on testicular dimensions.

MASTERS and FELS (1984) showed that testicular size is controlled by nutrition, even to the extent that well-fed rams in spring may have larger testes than poorly-fed rams in autumn. Nutrition appears to mediate its effect through increasing the frequency of pulses of LH and probably FSH (HOTZEL et al., 2003; BOUKHLIQ et al., 1997; LINDSAY et al., 1984; SUTHERLAND and MARTIN, 1980). The hypothalamo-pituitary system has been one of the most often studied by modern reproductive biologists. It has been suggested that reproductive axis does not seem to be closely linked with dietary intakes of amino acids or with circulating concentrations of glucose. However, the energetic components of the diet, particularly fatty acids, appear to play a key role in reproductive responses to changes in nutrition. Fatty acids can stimulate GnRH-dependent pathways that initiate changes in testicular function (BLACHE et al., 2002; BOUKHLIQ et al., 1997).

It has been revealed that poor maternal nutrition during pregnancy reduces the number of Sertoli cells in newborn lambs. The Sertoli cells are strong candidates for foetal programming of future performance, because the number of Sertoli cells is highly correlated with adult testicular size and the maximum rate of sperm production (ALEJANDRO et al., 2002). Likewise, our study demonstrated a significant correlation ($r = 0.96$) between SC and sperm production, which is a direct function of testicular size (AX et al., 2000).

Seasonal variations in testicular size and plasma testosterone concentration of rams have been reported in temperate areas (DUFOUR et al., 1984). These changes are related to photoperiod, so the change from long to short days stimulates both testicular development and testosterone secretion. The present study was carried out during the reproductive

period, from November to January, and therefore an effect related to photoperiod was not expected.

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, testosterone and inhibin (TJONDRONEGORO et al., 1996). However, more recent studies support an alternative pathway that promotes testicular growth in the absence of changes in gonadotrophins secretion-GnRH-independent pathway (BOUKHLIQ et al., 1997; BOUKLIQ and MARTIN, 1997; HOTZEL et al., 1995). FERNANDEZ'S results (2004) concerning the significant effect of protein supply diets on testicular size without affecting circulating testosterone concentrations would further support this hypothesis.

There is no study concerning the relationship between diet and semen volume. However, the results of the present study depicted that semen volume tended to be significant ($P = 0.073$) in treatment group than it was in the control group. Semen volume is one of the important factors in semen evaluation and reproduction performance in the males (AX et al., 2000).

A number of studies have demonstrated that the spermatogenesis in rams is sensitive to increases in protein intake. This effect has been related to an increase in testicular size because it is due to an increase in the volume of seminiferous epithelium and in the diameter of seminiferous tubules (HOTZEL et al., 1998; ABI SAAB et al., 1997; OLDHAM et al., 1978). The results presented in this work confirm that sperm production, as well as total number of spermatozoa per ejaculate, can be affected by improved diet. This result is consistent with the study of FERNANDEZ et al. (2004). The other ejaculate parameter (proportion of live and dead spermatozoa) measured in the current study was not affected by dietary treatment.

In conclusion, the results presented suggest that improved dietary intake above maintenance requirements affects scrotal size and increases sperm concentration significantly. However, semen volume and sperm viability percentage was not significantly affected. Therefore, it is strongly recommended that an improved diet be provided to Bakhtiary rams during the breeding season in order to improve their reproductive performance.

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SAŽETAK

Cilj istraživanja bio je utvrditi utjecaj poboljšane hranidbe na održavanje preporučljivih reproduktivnih pokazatelja uključujući veličinu sjemenika, količinu sjemena, koncentraciju i viabilnost. Dvanaest ovnova pasmine Bakhtiary podijeljeno je u dvije skupine po 6 ovnova i hranjeno različitom hranom u tijeku 12 tjedana, pripremljenom tako da se zadovolje uzdržne potrebe (suha tvar, energija i proteini). Vrijednosti za suhu tvar, ječam i sojino brašno za kontrolnu skupinu iznosile su 1,5 kg, 8%, 0%, a za pokusnu skupinu 2,1 kg, 25% i 17%. Sperma je uzimana pomoću umjetne rodnice svaki drugi tjedan. Obujam skrotuma, koncentracija spermija i njihov ukupan broj u ejakulatu značajno je porastao u pokusnoj skupini ($P<0,05$), dok obujam sperme te omjer između mrtvih i živih spermija nisu bili značajno promijenjeni ($P>0,05$). Postojala je značajna linearna korelacija između obujma mošnjice i proizvodnje spermija u ispitivanoj skupini ($P<0,01$, $r = 0,96$). Zaključeno je da poboljšana hranidba s višom razinom energije i nadomjescima proteina u Bakhtiary ovnova mogu poboljšati njihovu reproduktivnu sposobnost u sezoni parenja.

Ključne riječi: ovan, sperma, obujam skrotuma, hranidba