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EFFECT OF PROTECTED METHIONINE SUPPLEMENTATION ON BODY WEIGHT, TESTICULAR PARAMETERS, SEMEN CHARACTERISTICS AND TESTOSTERONE HORMONE OF AWASSI RAM LAMBS

Abdulnasir Th. Alkhashab, Falah. H.Al- Hassan, Samem. F. Al-dabbagh, H. W. Kassim and Thaer M. Abd Albaki
Animal production department, Faculty of agriculture and forestry, University of Mosul, Iraq
Email : drnasiralkhashab@gmail.com

ABSTRACT

The objective of this study was to evaluate the impact of feeding protected Methionine on body weight, testicular parameters, semen characteristics and testosterone hormone in growing Awassi ram lambs. Ten mal Awassi lambs, weighing 34 kg ± 0.5 kg, age 8 month, were randomly allotted into two groups of equal number, fist was control group(c) and the second was treatment group (T). Lambs fed concentrated ration which contained barely seeds, wheat brand, soybean meal, urea, salt, limestone and wheat straw as a roughage ration. Lams in T group fed the basal control ration with 5% rumen protected methionine/ head / day. Testicular parameters was recorded. Semen collected by electric ejaculator and volume, sperm motility, sperm concentration, live and dead sperms and sperm abnormalities were evaluated. The results showed a significant ($p \leq 0.05$) increase in live body weight reached 47 kg ± 0.509 kg in treatment group than 40.4 kg ± 1.326 kg, protected methionine had no effect on testicular parameters in the first and second months of experiment but it had a significant effect in the third month of experiment. No significant effect of protected methionine supplementation on fresh semen characteristics (Semen ejaculated volume, semen concentration, mass and individual motility) but alive sperm ratio increased and dead sperms decreased significantly ($P \leq 0.05$) in treatment group than control in last month of experiment. Testosterone concentration was significantly ($P \leq 0.05$) increased in the second and third month in treatment group than control. The dietary supplementation with protected methionine showed a significant effect on body weight, Testosterone level and slightly effective on testicular parameters, semen characteristics of Awassi ram lambs.

Keywords : Awassi lambs, protected methionine, testicular and semen parameters.

Introduction

All animals require essential amino acids, the building blocks of proteins, for optimal growth, reproduction, lactation and maintenance. Amino acids absorbed in the ruminant small intestine are derived from dietary and microbial proteins that are undegraded in the rumen. Methionine is often the most limiting amino acid in most diets and in microbial protein (Storm and Orskov, 1984; Buttery and D'Mello, 1994). Plant proteins deficient in methionine amino acid (Schwab *et al*., 1986) and ruminant animals cannot synthesize methionine, therefore methionine must be supplied from the dietary degradable protein and microbial protein synthesis (Lapierre *et al*., 2009). Ruminally-protected methionine (RPM (bypasses the ruminal degradation, because of the coating process, and enters the small intestine where it can be directly absorbed. However, the bioavailability of methionine is limited due to its degradation in the rumen (Schwab *et al*., 2001). So Supplementing ruminant diets with RPM can increase the flow of nitrogen and amino acids to the small intestine (Titgemeyer *et al*., 1989) and result in improved growth and efficiency of nitrogen utilization and consequently the general performance of ruminant livestock (Goedeken *et al*., 1990). In addition to, methionine is the most limiting amino acid for protein synthesis in growing lambs (Storm and Orskov, 1984; Nolte *et al*., 2004). But it is not clear whether supplementation of feed with Methionine can improve testicular measurements and semen characteristics of ram lambs. Nevertheless Nizza *et al* (2000) found that supplementation with methionine improved significantly the motility of rabbit semen and libido. The hypothesis that impaired testicular development could be rescued by methionine inriched diet has not yet been examined. The objective of this study was to determine the effect of dietary supplementation with methionine on the growth performance, testicular parameters, semen characteristics and serum testosterone concentration of Awassi ram lambs.

Materials and Methods

This experiment was carried out at animal field of animal production department, faculty of agriculture and forestry, University of Mosul. This study was conducted during June and august (2019) .Ten of adult fertile Awassi male lambs age from 10–12 months with average 34 kg ± body weight were used in present study . These lambs were supervised by veterinarians throughout the experiment. The animals were randomly divided into two equal groups (5 animals each) to perform T_1 control (C) and T_2 treatment group (T_2). The animals placed in two same pens (3×4m) under the same environmental condition and fed basic ration (Concentrate + Roughed). The basic ration in two groups

supplemented as 2.5% of LBW. The ingredients and chemical composition of ration are presented in Table 1, water and molds of mineral salts were available daily in front of animals, The ration was offered twice daily in equal quantities at 8:00 am and 3:00 pm .The lambs in group T_1 (control) were fed the basic ration only,whereas lambs in group T_2 were fed the basic ration (control) + 5% protected methionine of daily ration intake. Lambs were weighted at the beginning of the experiment and at the end of each month for calculating the total weight gain rate. The amount of basic ration were calculated according to NRC (2007). The chemical compositions of basic ration were analyzed according to AOAC (2007) methods and illustrated in (Table 1). The rumen protected methionine (Mepron M85R; Degussa Hulls AG, Hanau, Germany) used in this study is a methionine analogue that is physically protected by an ethyl cellulose and stearic acid film. Semen was collected by electrcoejaculater of sheep and goats from each lamb weekly during the duration of experiment, which lasted for 3 months.

Testicular dimensions, length, width of each testis after forcing against scrotum and scrotum thicknesses were measured with caliper. The scrotal circumference was measured by placing a measuring tape around the testis at the widest point (Ahmad *et al*., 2010).

Semen was evaluated immediately upon collection for general characters (semen ejaculated volume, concentration, mass motility, live, dead sperm and abnormal spermatozoa). Semen ejaculated volume (ml) was measured using a graduated collection tube to the nearest 0.1 ml.), Mass motility was tested by taking 10 µl of undiluted semen and placing on two places of warmed slide placed stage warmer (37◦C) and scoring on a scale of (0–5). Semen volume, mass motility and individual motility (0–100%) was estimated according to (Chemineau *et al.,* 1991) and sperm concentration was determined by Spectrophotometers according to (Salisbury *et al.*, 1978). The percentage of live, dead and sperm abnormality was calculated by method of (Chemineau *et al*., 1991). Serum testosterone concentrations were assayed in duplicate, using a commercial enzymatic immunoassay (ELISA) kit (DiaPlus Inc., USA).

Statistical analysis

The data were statistic analyzed using the statistical analysis system (SAS 2007). Separation among means was carried out according to Duncun Multiple Range Test (Duncan 1955). Data of body weight change, testicular and semen parameters were statistically analyzed according to the following model:

$$Yij = \mu + Ti + eij$$

Where :

yij = Represents observation .
µ = The general mean of studied character.
Ti = Effect of the studied treatments (experimental group).
Eij = Experimental error.

Table 1 : Ingredient and chemical composition of basal ration.

Ingredient*	%
Barley	70
Wheat bran	18
Soybean meal	7
Urea	0.5
Line stone	0.5
Salt	0.5
Barley straw	3.5
Chemical composition**	%
Crude protein	13.7
Dry matter	91.36
Organic matter	90.70
Ether Extraction	2.32
Crude fiber	7.96
Digestible Energy (K.kal./Kg)	2463

*Ingredient of ration according to (NRC, 2007).

** Chemical composition according to (A.O.A.C., 2007)

Results and Discussion

The result illustrated in table 2 showed a significant ($p \leq 0.05$) increase in final body weight reached 47.0 kg in treatment group than 34.0 kg in control group, the result in agreement with Kassim *et al.* (2020) which they found a significant increase in BW of lambs fed methionine and lysine with their dams in second and third lactation months, also Shaawi (2017) found a significant increase in final body weight of lambs fed diet supplemented with 5 gm /head / day methionine. Zhu (2010) found a significant effect of different levels of zinc methionine supplemented in the ration on body weight gain of lambs, also Lynch *et al.* (1991) noted significant increase in body weight when crossbreed blackface ewes and their lambs fed with protected methionine . But the results in contrast with Ren *et al.* (2019), El-Tahawy *et al.* (2015) who they found insignificant increase in body weight of lambs supplemented with 3.30 or 3.63 g methionine / kg concentrate feed mixture than control group. Also Belal *et al.* (2008), Mutassim *et al.* (2003) did not found any significant effect of methionine supplementation on final body weight of Awassi lambs and Rent *et al.* (2019) attributed the reason that they did not found a significant effect on the average body weight of lambs to their unprotected of methionine. The results are considered as good indicator of an improvement in the balance of amino acids in the metabolic protein and available amino acid profile when methionine was administrated (Movaliya, 2013).

The results about testicular parameters in table 2 showed that no effect of protected methionine supplementation on testicular parameters in fist and second months of experiment, while the effects became significantly ($p \leq 0.05$) evident in the last month of experiment (Third month) except scrotum thickness. This increase may be due to the positive effect of protected methionine on body weight of ram lambs and to the positive correlation between body weight and testicular parameters (Salhab *et al*., 2001; Foster *et al*., 1989; Alkass *et al*., 1987).

The result illustrated in table 3 showed that there was no significant effect of protected methionine supplementation on fresh semen characteristics (Semen ejaculated volume, semen concentration, mass and individual motility) but alive sperm ratio increased and dead sperms decreased significantly in treatment group than control in last month of experiment, also the result showed a significant decrease in sperms abnormality ratio in treatment group than control group in the last tow months. Although there was no significant effect of protected methionine but it was slightly improvement in this semen characteristics in treatment group

than control group. This results agree with Ezzat *et al* (2019) which found an un significant improvement in semen volume, concentration, individual motility but the researcher get un significant decrease in dead and total abnormal sperms in treatment group than control in male laying hens supplemented with zinc methionine than control group, also result in agreement with Nizza *et al.* (2000) which found un significant effect of methionine supplementation on evaluations of for volume, concentration, and little variation in motility and live spermatozoa percentage. But the results in contrast with Hong *et al.* (2018) which found a significant effect of methionine supplementation in ration on semen concentration, individual motility, live sperms and significant decreased in abnormal sperms percentage on boar, also our results contrast with El-Sharawy *et al.* (2012) which he found a significant effect of drenched zinc methionine on semen parameters of crossbred Rams. Testosterone hormone concentration illustrated in table (3) increased significantly ($p \leq 0.05$) in treatment group than control group in the second and third month of experiment. This result in agreement with Shamiah *et al*. (2017) who illustrated that supplementation of diet with (0.30 mg/ kg diet) selenomethionine increased significantly testosterone concentration of Cockerels, but in contrast with Zhai *et al.* (2016) who did not found significant effect of supplementation of diet with methionine on testosterone concentration of male broilers. This increase in concentration of testosterone revealed to that dietary supplementation with methionine improved growth and live body weight (LBW) and testosterone hormone was positively correlated with BW and the increase in growth and LBW may led to an increase in Testosterone concentration (Zhai *et al*., 2016; Ashton *et al.*, 1995).

Table 2 : Effect of methionine on body weight and testicular measurements of experimental Ram lambs (mean ± stander error).

Parameters	**T_1 control**		**T_2 methionine**
Initial body weight kg	34.600		34.00
Final body weight kg	40.400± 1.326 b		47.100± 0.509 a
Measurements	month	T_1 cont.	T_2 methionine
Left testes length cm	1	8.248 ± 0.315	9.518± 0.216
	2	8.734 ± 0.439	9.692± 0.232
	3	9.004 ± 0.570 b	11.000 ± 0.140 a
Right testes length cm	1	7.910 ± 0.329	9.130± 0.258
	2	8.908 ± 0.568	9.958± 0.301
	3	9.302b ± 0.797 b	11.910 0.135 a
Left testes depth cm	1	4.230± 0.153	4.784 ± 0.249
	2	4.444 ± 0.216	5.038 ± 0.263
	3	4.542 b ± 0.267 b	5.366 ± 0.146 a
Right testes depth cm	1	4.324± 0.209	4.710± 0.104
	2	4.710 ± 0.336	5.340 ± 0.060
	3	4.732 ± 0.302	5.266 ± 0.262
Scrotal circumference cm	1	23.400 ± 0.967	25.100 ± 0.509
	2	24.560 ± 1.359	27.600 ± 0.484
	3	24.300 ± 1.347 b	28.300 ± 0.974 a
Scrotum thickness cm	1	0.326 ± 0.013	0.422 ± 0.043
	2	0.336 ± 0.042	0.396 ± 0.014
	3	0.342 ± 0.034	0.368 ± 0.22

[a,b] Different superscripts within row differ significantly ($p \leq 0.05$).

Table 3 : Effect of methionine on fresh semen characteristics, testosterone levels of experimental Ram lambs (mean ± stander error)

Characteristics	**Month**	**T_1 concentrate**	**T_2 methionine**
Semen volume ml	1	0.933 ± 0.317	o.800 ± 0.173
	2	1.100 ± 0.437	1.000 ± 0.115
	3	0.733 ± 0.176	0.867 ± 0.176
Semen conct. × ^{9}10 /ejaculate	1	1.187 ± 0.190	1.333 ± 0.064
	2	1.294 ± 0.274	1.552 ± 0.182
	3	1.719 ± 0.250	2.066 ± 0.107
Mass motility (1 – 5)	1	3.167 ± 0.440	3.60 ± 0.115
	2	4.000 ± 0.115	4.233 ± 0.392
	3	3.400 ± 0.493	4.366 ± 0.384
Individual motility %	1	80.000± 2.886	82.000 ± 2.000
	2	83.333 ± 1.667	87.330 ± 2.667
	3	79.333 ± 2.333	88.340 ± 4.09
Live sperm %	1	84.333± 1.452	85.667 ± 0.667
	2	87.000 ± 1.732	89.333 ± 1.763
	3	85.000 ± 0.577 b	90.200 ± 1.154 a

Dead sperm %	1	15.667± 1.452	14.333 ± 0.666
	2	13.000 ± 1.732	10.667 ± 1.763
	3	15.000 ± 0.577 a	10.000 ± 1.154 b
Abnormal sperms %	1	5.667± 0.334	4.666 ± 0.332
	2	6.000 ± 0.577 a	3.333 ± 0.333 b
	3	6.333± 0.333 a	3.667 ± 0.330 b
Serum Testosterone ng/ ml	1	0.214 ± 0.188	0.478 ± 0.158
	2	0.257 ± 0.113 b	3.403 ± 0.389 a
	3	0.580 ± 0.109 b	1.713 ± 0.213 a

[a,b] Different superscripts within row differ significantly ($p \leq 0.05$).

Conclusion

Protected Methionine had positive effect on lamb body weight and slightly effective on testicular parameters and semen characters in growing Awassi ram lambs.

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