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Effect of adding protected methionine to the ration on hematological indices and biochemical blood parameters in Awassi lambs

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Abstract

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This experiment was undertaken to study the effect of protected Methionine supplementation on hematological and biochemical parameters in growing Awassi lambs. For that, ten male Awassi lambs 8 months old, with 34 ± 0.5 kg initial bwt, were randomly assigned to two groups of equal size, the first one represented as a control group(c) and the second was the treatment group(T). Lambs of control group were fed basal ration consisting of a concentrated diet consisting of barely seeds, wheat brand, soyabean meal, salt, urea, limestone and wheat straw as a roughage diet. The treatment group lambs were fed a basal control ration containing 5 g/ head/day (RPM) rumen protected methionine. Blood samples were collected once monthly and the experiment lasted 3 months. Results about blood parameters showed a significant ($P \leq 0.05$) decrease in WBC and a significant ($P \leq 0.05$) increase in blood glucose, cholesterol levels in methionine treated group than control group, but there are no significant effect of treatment with protected methionine on other hematological values and biochemical parameters. It concluded that supplementing the ration with protected Methionine led to non-significant effect on hematological and biochemical values except WBC, glucose and cholesterol in growing Awassi lambs.

Keywords: Awassi lambs; protected methionine; hematological and biochemical parameters

Introduction

The nutritional status of farm animals plays an important role in controlling the productivity and reproductive performance. The crude protein from the meal is digested in the rumen into a combination of amino acids, ammonia and peptides, which supply the majority of amino acids to the small intestine (Iburg & Lebzien, 2000). It can change the status of amino acids in rumen small intestine by feeding them foods of varying degradability and generating conditions which allow to increase the rate of passage of amino acids through the rumen by adding protected or by pass protein supplements into diets (Faichney, 1986). Methionine is an important amino acid that frequently serves as the first limiting amino acid in the synthesis of tissue protein in growing ruminants (Merchen & Titgemeyer, 1992), and methionine might be the limiting amino acid which restrict the nitrogen utilization when it is deficient in diets (Shan et al., 2007). In addition to that, methionine plays a crucial function in the antioxidant system by readily reacting with oxidants to create methionine sulfoxide (Livine et al., 1999), and it must be available at diets in sufficient quantities because it cannot be formed in the body (Richardson & Hatfield, 1978). Some blood hematological and biochemical parameters as indices of metabolic status were used for this purpose, taking into account the functional roles of amino acids in growth, general metabolism and health (Liker et al., 2005). Ruminant Blood metabolites and hematological values can be influenced by feeding rumen protected methionine (RPM) and methionine supplementation to the diet increased the performance of blood metabolites and hematological values of animals due to high nutrient value of protected methionine (Abdel- Gani

et al., 2011; Rodriquez – Guerrero et al., 2018). Maty (2021) and Liker et al. (2005) found that supplementation of protected methionine to diet had a significant effect on some blood biochemical and hematological values in blood serum of calves or beef cows respectively, while Shaawi (2017) found no evidence of methionine having a substantial influence on blood biochemical parameters (glucose, triglyceride, total protein, globulin) as well as hematological indices (RBC account, WBC and PLT account).

Therefore, the present study was planned to investigate the effect of adding protected methionine to diet on some hematological and biochemical blood parameters in Awassi lambs.

Material and Methods

This experimental work was done on a farm of animal production department located in college of agriculture field, University of Mosul. Ten of Awassi male lambs age 8 months old, 34 ± 0.60 kg bwt, were used in this work. The animals were randomly divided into two equal groups of five lambs each. The first group was kept as a control group (C) and the second was a treatment group (T). The animals were housed in two semi-open pens under the same environmental conditions. After an adaptation period of 15 day, lambs fed 1.2 kg dry matter/day/ head of basal ration consist of (Concentrate+Roughage) mixture. The amount and chemical analysis of basal ration are shown in Table 1 and calculated according to (Khawaja et al., 1978), and samples were taken for chemical analysis according to (AOAC 2007). The food meal was offered in equal quantities twice daily at 8:00 Am and 3:00 Pm. The lambs in C group were fed the basal diet without addition of methionine, whereas lambs in T group were received the same basal ration supplemented with 5 g/ head/ day protected methionine of daily diet intake. Blood samples from all experimental animals were obtained before morning feeding and the blood samples were collected via jugular vein puncture at monthly intervals. Blood samples as (10 ml) was drawn and divided into two parts, the first (2 ml) is placed in plastic tubes containing an anticoagulant (EDTA)for the purpose of using it for hematological blood tests (Hemoglobin Hb, Red blood cells RBC, White blood cells WBC, Lymphocytes, Neutrophil, Eosinophil, Monocytes , Mean corpuscular hemoglobin MCH, RBC, Mean corpuscular hemoglobin concentration MCHC, Mean corpuscular volume MCV and Packed cell volume PCV) .The second part of the blood sample (8 ml) were placed in glass tubes and the serum was separated by centrifugation at (3000 cycles/min) for 15 min, after which the serum is kept in the freezer (-20°C), up to subsequent analysis for measuring of biochemical parameters (glucose, total cholesterol, triglyceride, total protein, albumin, globulin, creatinine, urea, High density lipoprotein HDL, Low density lipoprotein LDL, Very low density lipoprotein VLDL, Aspartate aminotransferase AST, Alanine aminotransferase ALT).

Table 1. Ingredient and chemical composition of basal ration

Ingredient*	Percentage
Barley	70
Wheat bran	18
Soya bean meal	7
Urea	0.5
Limestone	0.5
Salt	0.5
Wheat straw	3.5
Chemical composition**	%
Crude protein	13.7
Dry mater	91.36
Organic mater	90.70
Ether Extract.	2.32
Crud fibers	7.96
Met. Energy, Kcal./kg	2463

* Ingredient of diet were taken from (NRC, 2007).
** Chemical composition calculated according to (A.O.A.C., 2007)
Met. energy of diet was calculated according to Alkhawaja (1978).

Hematological and biochemical analysis of blood plasma

Hematological parameters were analyzed by automatic hematology analyzer (Mytic18) from Orphee Swiss company, which it works by electrical impedance system. All biochemical parameters were analyzed by using Spectrophotometer (Emclab) German origin, which analyzes the chemical elements with optical spectrum technology and using commercial kits. All blood samples were analyzed in a special laboratory for analyzers.

Statistical analysis

Complete random design CRD were used for analysis of experimental data. Statistical analysis and comparisons between means were carried out using (SAS 2007). The significance of the tests between the means was tested using (Duncan, 1955). Data of blood parameters were statistically analyzed according to the following model:

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

where:

yij = Any observation
μ = Overall mean
Ti = Effect of treatment (experimental group)
Eij = Experimental random error.

Results

Hematological indices

In current study, the hematological indices of lambs in control group and RPM treated group during the trial are shown in (Table 2). The rumen protected methionine RPM did not affect significantly on hematological values (Hb, RBC, Lymphocytes, Neutrophil, Eosinophil, Monocytes, MCH, MCHC, MCV and PCV) of lambs in T group than control group, except WBC counts which the results showed that the treatment with rumen protected methionine RPM lead to a significant ($p \leq 0.05$) decrease of $4.7\times^{3}10/\mu l$ in treated group than $6.7\times^{3}10/\mu l$ in control group.

Table 2. Effect of rumen protected methionine hematological values of lambs in experimental groups (LS means ± SE)

Item	Control group	Methionine group
Hb, g /dL	11.20 ± 0.744	10.23 ± 0.493
WBC ($\times^{3}10$ / µL)	6.7 ± 2.948[a]	4.7 ± 6.022[b]
RBC ($\times^{6}10$ /µL)	10.84 ± 0.660	11.45 ± 0.371
Lymphocyte, %	66.30 ±4.821	66.33 ± 4.68
Neutrophil, %	31.23 ± 2.31	30.68± 2.22
Eosinophil, %	1.10 ± 0.98	1.36 ± 1.32
Basophil, %	0.82 ± 0.17	0.78 ± 0.19
Monocyte, %	0.94 ± 3.350	1.00 ± 2.085
MCH, pg	9.40 ± 0.152	9.76 ± 0.185
MCHC, %	36.57 ± 0.491	37.30 ± 0.604
MCV, fl	25.73 ± 1.23	26.26± 1.33
PCV, %	32.42±0.19	33.01±0.12

Note: Hb = Hemoglobin , WBC = white blood cells, RBC = Red blood cells, MCV = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV= mean corpuscular volume, PCV = Packed cell volume.

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

Table 3. Effect of protected methionine on blood biochemical parameters of lambs in experimental groups (LS means ± SE)

Biochemical parameters	Control group	Methionine group
Glucose, mg/dL	66.00± 3.506[b]	83.33 ± 3.565[a]
Cholesterol, mg/dL	65.00 ± 1.154[b]	87.00 ± 6.11[a]
Triglycerides, mg/dL	53.33 ± 4.302	52.33 ± 9.073
Total Protein, g/dL	6.73 ± 0.920	7.10 ± 0.100
Albumin, g/dL	3.87 ± 0.375	4.46 ± 0.120
Globulin, g/dL	2.86 ± 0.589	2.63 ± 0.033
Creatinine, mg/dL	1.55 ± 0.121	1.46 ± 1.220
Urea, mg/dL	6.93 ± 0.317	8.70 ± 0.838
HDL, mg/dL	37.66 ± 0.577	40.00 ± 1.452
LDL, mg/dL	44.00 ±5.686	39.33 ± 0.881
VLDL, mg/dL	11.00± 0.577	10.00 ± 2.081
AST, IU/L	123.00± 9.038	130.67 ± 14.774
ALT, IU/L	19.00 ± 4.509	29.66 ± 1.201

Note: HDL = High density lipoprotein; LDL= Low density lipoprotein; VLDL = Very low density lipoprotein; AST = Aspartate Transaminase; ALT= Alanine Transaminase.

[a,b]Different superscripts within the same row differ significantly at ($P < 0.05$)

Blood biochemical parameters

Results about blood biochemical parameters (Table 3) showed a significant ($p \leq 0.05$) increase in blood plasma glucose in treated group than control group, where the level of glucose increased to 83.33 mg/dl in protected methionine group than 66.00 mg/dl in control group. Moreover, the result revealed a considerable ($p \leq 0.05$) increase in total cholesterol level in blood plasma in treated group which reached 87.00 mg/dl than 65.00 mg/dl in control group. But the results showed no significant effect of RPM on the other biochemical parameters which studded in this experiment.

Discussion

The present study about hematological indices found that application of 5g/head/day protected methionine to ration resulted a significant decrease in WBC count (Table 2). This results agree with Maty (2021), who had a significant decrease in WBC in the methionine treated group than control in calves. This change in the number of leukocytes in methionine group than control group may be due to the metabolic outcomes of Methionine metabolism, which serves as a preservative and supportive ingredients for immunity and this effect of RPM could lead to a reduction in infection with various microbes (Ratika et al., 2018; Grimble, 1994). The result in contrast with (Shaawi, 2017; Liker et al., 2006) who did not found any significant change in WBC in lambs or beef cattle treated with methionine. While the results showed no significant detected on other hematological indices, and this result agree with Roland et al. (2014) and Liker et al. (2006).

The results about blood biochemical parameters revealed that adding protected methionine to diet resulted an increase in glucose and cholesterol levels in treated group (Table 3). Similar finding observed by (Kassim et al., 2019; li xue-ling et al., 2017; Sun et al., 2016), but these result contrast with Rodriquez – Guerrero et al. (2018) and Shaawi (2017). Also, the result in (Table 3) showed an increased in cholesterol level in treated group than control group and this finding agree with the results of Maty (2021), Rodriquez – Guerrero et al. (2018) and Alharthi et al. (2018), while contrast with Sun et al. (2016) and Davidson et al. (2008). This increase in glucose and cholesterol levels in treated group may be due to glucagon hormone increases, because a variety of amino acids have

the ability to increase hormone production and secretion (Kuhara et al., 1991). The amount of protein intake and the infusion of amino acids will led to an increased in the secretion of glucagon hormone (Peret et al., 1981), and the glucagon hormone stimulates the livers glucose synthesis, reducing the insulin- glucagon ratio and increase blood glucose and cholesterol levels can explained by glucagon increase (Ganong, 2005). Under the effect of glucagon, it seems that glucogenic amino acids appear to be converted to glucose in the liver, and there was a significant relationship between portal glucagon release and hepatic total amino acids elimination (Lapierre et al., 2000). Total protein, albumin, globulin, triglyceride, urea and creatinine levels had no effect by adding rumen-protected methionine. These results did not agree with Rodriquez–Guerrero et al. (2018) and Rajwade (2019), but it contrast with the results of Kassim et al. (2019); Kabu & Civelek (2012).

Plasma HDL tented to be in significantly higher in group T than C group, but LDL and VLDL were in significant reduced in T group than C group, similar finding observed with Kabu & Civelek (2012); Imik & Gunlu (2011) and Davidson et al. (2008) who showed no effect of methionine on HDL, LDL and VLDL values. The activity of ALT, AST, in blood plasma throughout the experiment were insignificant between T group and control group (Table 3), these results in agreement with Boraei (2018); Nazem & Askari (2013), but contrast to that, Kassim et al. (2019), who observed a significant reduced in AST activity in protected methionine-lysine treated groups in Awassi ewes at flushing period than control, while Kassim et al. (2019) and Liker et al. (2006) observed an increased in ALT activity in methionine treatment than control group.

Conclusion

Our study concluded that supplementing the ration with protected Methionine led to no significant effect on hematological and biochemical values except WBC, glucose and cholesterol in growing Awassi lambs without any effect on lambs health.

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