# Effect of addition protected amino acids (Methionine and Lysine) to the ration on some blood pictures of Awassi lambs

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## ABSTRACT

This study was conducted in fields of animal production / College of Agriculture and Forestry : University of Mosul for the period from 15/11/ 2021 - 15/2/ 2022, and the aim was to know effects of addition protected methionine and lysine on some blood pictures of Awassi lambs.The initial weight of lambs was about 30.85-31.30 kg with ages 10-12 months where there were randomly distributed to four treatments at a rate of five lambs for each treatment . The treatments were as follows, first treatment(control)T1 : as abasal diet, second treatment T2: added 1.5gm protected methionine/head/day to the basal diet, third treatment T3: added 3gm protected lysine/head/day to the basal diet, fourth treatment T4: added (1.5gm protected methionine + 3gm protected lysine/head / day to the basal diet and the treatment lasted 90 days, the protein content in the ration was 13.76%, and the energy level was (2520) kilocalories/kg of feed. The results of the statistical analysis showed a significant decrease ( $P\leq0.05$) in the number of white blood cells for the secondT2, third T3 and fourth T4 treatments compared to the control group T1 and a significant increase ($P\leq0.05$) in the concentration of globulin, cholesterol and triglycerides, and a decrease in urea and creatinine concentration in the fourth treatment T4 compared with the control treatment T1 and a significant increase ($P\leq0.05$) in albumin concentration in the addition treatments compared to the control.

key word: Awassi lambs, protected methionine, protected lysine, blood picture

## INTRODUCTION

To improve the efficiency of protein utilization , to requires to be balanced ration for specific Amino acid (AA),Microbial or dietary protein are often insufficient source of lysine and methionine to meet the requirement of fast growing animal ( 22 ) and it must be available at diets in sufficient quantities because they cannot be formed in the body ( 24) . Amino acid play an important role in regulation of the metabolic pathways essential for growth , Immunity ,body weight in animals ( 14 , 11) . Lysine and methionine have been identified as the two most common limiting AA in diets , methionine is the first limiting amino acid for synthesis of tissue protein in growing ruminants ( 16 ) As for these two acids, when used in a protected manner, can cross the rumen without decomposing and to enter the small intestine, where they are absorbed and improve growth and nitrogen retention, thus improving production performance(18). Blood metabolites and hematological indices of ruminants can be affected by feeding rumen protected methionine and the supplementation of methionine had appositive impact on blood metabolites performance and hematological indices of animals due to high nutrient value of protected

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methionine ( 1 , 20 ). So that methionine acts as a precursor amino acid for glutathione and plays avital role in detoxification(23) and also plays an important role in the antioxidant defense system by readily reacting with oxidants to form methionine sulfoxide ( 13 ), due to limited base line data of rumen protected methionine(RPM) and rumen protected lysine (RPL),the experiment has been studied to evaluated the effect of dietary supplementation of Rumen protected methionine ( RPM) and Rumen protected Lysine( RPL) on hematological and blood biochemical profile in Male Awassi Lambs.

## Materials and Method

Blood samples were drawn from lambs with a quantity of 10 ml at the end of the experiment from the jugular vein area using a plastic syringe capacity 10 ml and according to what was reported by ( 9) five hours after feeding and divided into two parts, the first 2 ml was placed in plastic tubes A container containing the anticoagulant Ethylene Diamin Tetra Acatic (EDTA) for the purpose of using it to perform blood tests (WBC, RBC, PCV, Hb). As for the second section of blood 8 ml, it was placed in test tubes and transferred directly to the laboratory. The serum was separated from the samples in a centrifuge at 3000 rpm for 15 minutes, and the resulting serum was kept in plastic containers under freezing (-20) degrees Celsius until biochemical tests were performed. After that, the characteristics (total protein, albumin, globulin, glucose, urea, triglycerides, cholesterol, ALT, AST, creatinine) were measured using the analysis kit, i.e. this Biolabo kit and using a Spectrophotometer of German origin manufactured by EMCLAB Company .

**Table (1) Ingredients and chemical composition of the standard diet.**

| **Feed material** | **from the deit %** |
|---|---|
| barley | 61.5 |
| wheat bran | 22 |
| yellow corn | 5 |
| soybean meal | 5 |
| hay | 5 |
| urea | 0.5 |
| salt | 0.5 |
| limestone | 0.5 |
| **chemical composition** | |
| dry matter | 92.12 % |
| organic matter | 95.77 % |
| crude protein | 13.76 % |
| crude fat | 2.08 % |
| raw fiber | 7.02 % |
| metabolic energy | 2520 Kcal/ kg |

* The components of the diet were estimated from the nutritional compounds in the laboratory according to what was reported ( 4), except for the energy, which was calculated mathematically from ( 3 ).


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## STATISTICAL ANALYSIS

The experiment is analyzed according to the system of simple experiments using the complete random design (CRD), and the comparison between the means is done to determine the significance using the multinomial analysis of means (5) and according to the following mathematical model: $Y_{ij}=\mu+t_i+e_{ij}$ $Y_{ij}$ = viewing value for the studied trait $\mu$= overall average of all $t_i$ = Effect of adding of protected Methionine and Lysine in fattening rations of Awassi lambs $e_{ij}$ = empirical error value of the experimental unit.

## Results and Dissection

The statistical analysis of the results in table (2) shows that the addition of PM (1.5 gm/ head/day) and PL ( 3gm /head/day ) and the addition of (1.5gm PM + 3gm PL /head /day) did not significantly affect the level of glucose and total protein in the blood compared to the control treatment, The results indicated a significant increase ( $P\leq0.05$) in the albumin concentration at groups treated with protected methionine or protected lysine or ( protected methionine + protected lysine) compared to the control group This may explain that giving these protected amino acids causes an increase in the absorption of real protein in the small intestine when these protected amino acids are given . The results of this study are in agreement with what was found ( 8), where the administration of a mixture of protected methionine at a dose of 3 gm/head/day and protected lysine at a dose of 20 gm/head/day led to a significant increase ( $P\leq0.05$ ) in the albumin concentration compared with the group control. The results of this stady did not agreement with what was found ( 6 ) where there were no significant differences in the concentration of albumin when giving Egyptian Barki lambs 3 gm/head/day of protected lysine in the forage compared to the control group. We also note that the level of globulin in the blood increased significantly in the fourth T4, third T3and second T2 treatment compared to the first treatment T1 (control) where it reached ( 25.00, 32.00, 32.80, 41. 00) g/100 ml respectively . The results of this study were consistent with the results( 8) where they noticed that giving protected methionine at a dose of 3 gm/head/day and proteced lysine at a dose of 20 g/head/day to cross bred calves led to a significant increase ( $P\leq0.05$) in globulin concentration compared with the control group. While the results of this study did not agree with the findings of ( 21) who concluded that giving crossbred calves protected methionine 2 gm/head/day and protected lysine 17 gm/head/day did not cause significant differences in globulin concentration compared with the control group


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**Table (2) Effect of treatment with protected methionine and Lysine on blood biochemical indicators of Awassi Lambs**

| Characters Treatment | Glucose mg/100ml | Total protein gm/100ml | Albumin gm/100ml | Globulin gm/100ml |
|---|---|---|---|---|
| **$T_1$: Control** | 6.60 a9 ± 1.80 | 64.40 a ± 1.32 | 31.60 b ± 0.74 | 25.00 c ± 1.41 |
| **$T_2$:protected Methionine 1. 5 gm** | 94.40 a ± 2.29 | 71.80 a ± 1.01 | 36.00 a ± 1.18 | 32.00 b ± 1.41 |
| **$T_3$:protected Lysine 3 gm** | 100.60 a ± 1.86 | 67.80 a ± 1.62 | 35.00 a ±1.55 | 32.80 b ± 0.73 |
| **$T_4$:combination protected (methionine 1.5gm gm Lysine )** | 95.80 a ± 2.15 | 69.40 a ± 1.57 | 38.40 a ± 0.81 | 41.00 a ± 2.64 |

The averages with different letters within the same column indicate the presence of significant differences at ($p \leq 0.05$).

Table (3) shows that the level of cholesterol in the blood was significantly increased ( P≤0.05) in the treatments added , as it was superior in the fourth treatment T4 (added PM 1.5 gm/ head/day+ PL 3gm/ head /day ), and the third treatment T3 (added PL 3gm/ head/day) and the second treatment T2 (added PM 1.5gm /head/day) to the diet compared with the control treatment T1(without addition), where the levels of cholesterol in blood was (52.80, 70.40,74.60, 88.00) mg/100ml, respectively. The reason is attributed that Protected methionine increases the secretion of glucagon, which stimulates the secretion of growth hormone, which raises cholesterol(12) ,the level of triglycerides increased significantly ( P≤0.05) in the fourth T4, third T3and T2 second addition treatments compared with the controlT1 , reaching ( 10. 60, 20. 40, 30. 60, 48. 40) mg/100ml respectively . The results of this study are in agreement with the findings of ( 15), where giving fattening calves protected methionine at a dose of 15 gm/head/day led to an increase in the concentration of cholesterol and triglycerides . The results of this study also agreed with what was found by ( 20) that giving lambs 1.5 gm of protected methionine/head/day caused a significant increase (p≤0.05) in the concentration of triglycerides compared with the control group. The results of this study did not agree with the findings (10), where giving Awassi ewes 2.5 gm protected methionine and 2.5 gm protected lysine/head/day led to a significant decrease ($p \leq 0.05$). in the concentr-ation of triglycerides compared with the control group. Also, the results of this study did not match the findings of ( 22), where giving cows 15 gm of protected methionine/head/day led to a decrease in cholesterol concentration compared to the control group. We note from the same table, that the level of creatinine significantly decreased(P<0.05) in the fourth treatmentT4 (added protected methionine 1.5gm/ head/day +protected lysine 3gm/ head/day ) compared with the control treatment T1, while we note that the second and third treatment did not differ significantly compared with the


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control and the values were (0.698, 0.562, 0.546, 0.416) m g/100ml respectively As for the absence of a significant difference in the concentration of AST and ALT between the groups in this study, this indicates that the protected methionine and lysine have no negative effects on the liver, and this is consistent with what was found (17) where they mentioned that giving Iranian Cashmere goats 3 gm of protected methionine / head / day there were no significant differences in the concentration of ALT and AST. The results of this study also agreed with what was found by ( 26) where the administration of protected methionine to Barki sheep at a dose of 7 gm alone or with protected lysine at a dose of 3 gm / head / day did not lead to significant differences in the concentration of AST and ALT compared to with a control group. The results of this study also agreed with what was found (27) that giving protected methionine at a dose of 15 gm/head/day to Barki ewes did not cause significant differences in the level of AST and ALT concentrations compared with the control group. The results of this study did not match what was found ( 10 ), where giving Awassi ewes 2.5 gm protected methionine and 2.5 gm protected lysine / head / day caused a significant decrease ($p \leq 0.05$) in the concentration of AST enzyme and a significant increase ($p \leq 0.05$) In ALT enzyme compared with the control group . with regard to the concentration of urea and its decrease in the groups treated with protected amino acids compared to the control .The decrease in urea when given protected methionine acid is explained by the fact that this acid improves the balance of amino acids in the metabolized protein and thus the lack of process Removing the amine from the absorbed acids ( 7) and the high absorption of the protected amino acids and increasing their use in tissue growth works to reduce the level of urea in the blood. The results of this study agreed with the findings of (2) where giving the Arabian lambs 5gm protected methionine or 5 gm protected lysine/head/day or both led to a significant decrease ($p \leq 0.05$) in the concentration of urea Compared with the control group, the reason may be due to the increase in protein absorption and deposition in tissues, and the reduction in the catabolism of amino acids, and thus to the low concentration of urea ( 19 ). Also, the results of this study agreed with what was found by ( 12) that giving cows protected methionine at a dose of 15 gm/head/day for 94 days led to a decrease in urea concentration compared to the control group. The results of this study differed with what was found by ( 24), where the administration of protected methionine at a dose of 18.2 gm/head/day and protected lysine at a dose of 11.7 g/head/day together to Holstein cows caused a significant increase($p \leq 0.05$) in urea concentration at Comparing it with groups that took protected methionine alone or protected lysine alone or the control group with regard to the concentration of creatinine and its decrease in the groups treated with protected amino acids compared to the control, it may explain the decrease in the level of creatinine when giving protected amino acids because these acids are considered building components of protein, so the breakdown of muscle protein does not occur, thus reducing creatinine. The results of this study supported what(26 ) whereby found giving Barki ewes of 3 gm protected lysine / head / day or 7 g protected methionine / head / day caused a significant decrease in creatinine concentration compared to With the control group. The result of


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this study also agreed with the findings of ( 19), where there was a significant decrease in the concentration of urea and creatinine when giving 5 gm protected methionine /head/day and 15 gm protected lysine/head/day for Sachiwal type calves compared to With the control group, the reason may be the increased absorption of these protected acids in the small intestine and their effective use in building tissues. The decrease in the concentration of urea and creatinine in this study when giving protected amino acids (methionine+ lysine), it may be due to the low concentration of ammonia during bacterial fermentation in the rumen of lambs that took these protected amino acids and consequently the lack of ammonia absorbed from the rumen wall, which turns into urea in the liver. The low level of ammonia in the rumen of sheep fed on protected amino acids is a reflection of the low level of urea in the body (1).

**Table ( 3 ) Effect of treatment with protected methionine and lysine on blood biochemical indicators of Awassi lambs**

| Characters / Treatment | Cholest- er mg/100ml | Trigly-ceri mg/100 ml | Urea mg/100 ml | Creatini-ne mg/100ml | ALT IU/l | AST IU/l |
|---|---|---|---|---|---|---|
| **$T_1$: Control** | 52.80 c ± 1.98 | 10.60 d ± 0.24 | 84.80 a ±1.77 | 0.698 a ± 0.11 | 14.00 a ± 1.78 | 39.40 a ±1.28 |
| **$T_2$:Protected Methionine 1.5gm** | 70.40 b ± 2.90 | 20.40 c ± 2.74 | 65.40 b 2.42 | 0.562ab ±0.60 | 15.80 a ±1.80 | 40.20 a ±1.39 |
| **$T_3$:protected Lysine 3 g/ml** | 74.60 b ± 2.35 | 30.60 b ± 3.37 | 51.60 b ±11.54 | 0.546ab ±0.042 | 16.40 a ±1.72 | 38.00 a ±1.30 |
| **$T_4$ : combination protected (methionine1.5gm Lysine 3gm )** | 88.00 a ±2.54 | 48.40 a ± 3.29 | 61.60 b ± 1.02 | 0.416 b ± 0.025 | 15.20 a ±1.6 | 38.80 a 1.82± |

The averages with different letters within the same column indicate the presence of significant differences at ($p \leq 0.05$).

Table (4) It is clear from the results of the statistical analysis recorded in Table ( 4) that there are no differences Significant ( $p \leq 0.05$) in hemoglobin concentration, red blood cell count and packed cell volume among the four experimental treatments. It was also shown that there was a significant decrease ( $p \leq 0.05$) in white blood cells in the fourth groupT4 ( protected methionine + protected lysine) 8.640, the third group T3 (protected lysine) 9.260 and the second groupT2 (protected methionine) 9.540 compared with the control group T1 10.330. These results are in agreement with what was found by the researcher (2), where he did not find any significant differences in hemoglobin concentration, packed cell volume, and erythrocyte count among the four experimental groups (control, 5 gm PM , 5 gm PL, 5 gm PM + 5


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gm PL ). Also, the results of this study agreed with what was found ( 19 ) where there were no significant differences in the hemoglobin concentration, the number of red blood cells and the packed cell volume when giving Sachiwal Calves protected methionine at a dose of 5 gm/head/day and/or the protected lysine at a dose 15 gm/head/day compared with the control group. The result of this study did not agree with what was found by ( 10), where there was a significant increase ($p \leq 0.05$) in the number of red blood cells in fattening calves that took 15 gm of protected methionine compared with the control group.Also the results of this study also did not agree with findings of ( 2 ), who did not obtain significant differences in the number of white blood cells when giving lambs protected methionine alone or with protected lysine at a dose of 5 gm/head/day for each of them compared with control group.

**Table (4) Effect of protected methionine and lysine on blood images of Awassi lambs**

| **Characters**<br><br>**Treatment** | **White blood count** $\times 10^3$ | **Red blood count** $\times 10^6$ | **Hemoglobin lev g/100ml** | **PCV %** |
|---|---|---|---|---|
| **$T_1$: Control** | 10.330 a ± 1.94 | 12.32 a ± 0. 97 | 12.14 a ± 0.64 | 36 a ± 0.019 |
| **$T_2$ :Protected Methionine 1.5 gm** | 9.540 bc ± 2.41 | 12. 62 a ± 0. 61 | 12.04 a ± 0.71 | 35 a ± 0.018 |
| **$T_3$: protected Lysine 3 gm** | 9.260 b ± 2.72 | 13.1 4 a ± 0. 53 | 12.70 a ± 0.36 | 38 a ± 0.010 |
| **$T_4$: combination protected (methionine 1.5gm Lysine 3 gm)** | 8.640 c ± 1. 73 | 12. 43 a ± 0.607 | 12.28 a ± 0.23 | 37 a ± 0.004 |

The averages with different letters within the same column indicate the presence of significant differences at ($p \leq 0.05$).

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