EFFECT OF DIETARY PROTECTED LYSINE SUPPLEMENTATION ON MILK YIELD, COMPOSITION AND SOME BLOOD AND RUMEN PARAMETERS IN LOCAL EWES

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ABSTRACT

This study was aimed to evaluate the effect of supplementing rumen protected lysine on milk production, composition and some blood and rumen parameters. Nine local ewes were assigned into three groups (3 ewes for each) and were allocated into 3x3 Latin-square design. The first group (control) was fed concentrate diet. The second group (F-LYF) was fed the control diet and supplemented with free lysin (3g/ewe/day). The third group (P-LYS) was fed the control diet and supplemented with rumen protected lysine (3g/ewe/day). The ewes were offered 1000 g/ewe/day of concentrate and wheat straw *ad libitum* in addition to grazing for 6 hr per day. Results revealed that ewes supplemented with P-LYS and F-LYS had a non-significant (P>0.05), increase in daily milk yield, fat content and yield, protein yield, fat corrected milk (FCM) and energy corrected milk (ECM). The serum urea concentration was reduced significantly (p<0.05) in the (P-LYS) and (F-LYF) compared to control group, while neither protected nor free lysine affecting (P>0.05) all blood parameters as well as rumen pH and protozoa numbers, while ammonia nitrogen (NH_3-N) was numerically increased (P>0.05).

Key words: concentrated diet, wheat straw, corrected milk

مجلة العلوم الزراعية العراقية -2022 :53(6):1317-1324 مصطفى

تأثير اضافة اللايسين المحمي الى عليقة النعاج المحلية في انتاج الحليب ومكوناته وبعض قياسات الدم وسائل الكرش

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المستخلص

تهدف الدراسة تقييم تأثير إضافة اللايسين المحمي في انتاج الحليب ومكوناته وبعض قياسات الدم وسائل الكرش في النعاج المحلية. تم توزيع تسعة نعاج محلية الى ثلاث مجموعات (٣ نعاج لكل مجموعة) باستعمال تصميم المربع اللاتيني (٣×٣). غذيت المجموعة الأولى على العليقة المركزة. وغذيت المجموعة الثانية على عليقة السيطرة بإضافة ٣غم من اللايسين الحر وغذيت المجموعة الثالثة على عليقة السيطرة مع إضافة ٣غم من اللايسين المحمي. غذيت النعاج العليقة المركزة (١٠٠٠غم/رأس/يوم) مع تبن الحنطة حد الشبع فضلا عن الرعي لمدة 6 ساعات يوميا. بينت النتائج ان تغذية النعاج على اللايسين المحمي والحر أدى الى زيادة غير معنوية (P>0.05) في حاصل الحليب اليومي، نسبة دهن وبروتين الحليب والحليب المعدل للدهن وطاقة الحليب المعدلة. انخفض يوريا مصل الدم معنويا (p<0.05) في دم النعاج المغذاة على اللايسين المحمي والحر مقارنة بمجموعة السيطرة. بينما لم تؤثرفي جميع الصفات البايوكيميائية ودرجة الحموضة في سائل الكرش وعدد البروتوزوا بينما وجد زيادة غير معنوية (P>0.05) في امونيا الكرش.

الكلمات المفتاحية: اللايسين، النعاج المحلية، حاصل الحليب، قياسات الدم والكرش.

Received:22/3/2021, Accepted:27/6/2021

INTRODUCTION

It is known that amino acids are the building blocks of protein, so they have versatile and an important physiological function beyond their roles (52). In growing animals, for synthesis of tissue protein (31), and for milk protein synthesis (7; 34). Proteins, specifically amino acids profiles, are the most limiting nutrient for milk production in ruminants with high genetic merit. Thus, ruminant performance can be improved by dietary supplementation with rumen protected amino acids (RPAA), mainly methionine (Met) and lysine (Lys) (5). Recently, studies have been carried out in order to identify the limiting amino acids in milk production, in cows and ewes (6), as well as in goats (19). Furthermore, it was found in ruminants, that methionine and lysine are generally recognized as the first or second limiting amino acid, which will restrict the nitrogen utilization when it is deficient in the diets (43) as well as in dairy ruminants for milk protein synthesis as they constitute building blocks for caseins synthesis (7; 34). Studies revealed that ruminant animals fed on poor quality forages with inadequate protein showed better performance with supplementation of quality protein or rumen protected amino acids particularly methionine and lysin (3). Also, it was indicated that methionine is an essential amino acid limiting in most dietary situations for production in dairy cows fed diets based on corn grain and soybean meal (9; 53), as well as in ewes and goat (19). Information in literature on Iraqi local ewes fed with rumen-protected amino acids is scarce therefore, the main objective of this study was to investigate the effect of PLYS (protected lysine) on milk production, composition and some rumen and blood parameters in local ewes compared to free lysine (lysine-HCl) as positive control and no lysine as negative control.

MATERIALS AND METHODS

The present experiment was conducted at the animal farm, Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok during the lambing season 2021. Nine lactating local ewes weighing (40.81±1.27 kg) were used in a 3 × 3 Latin square design with three periods of 15 days each. Following one week adaptation period, animals were assigned to three treatments. The first group (Control) no lysine as negative control was fed on basal diet (TMR) consist of 50% barley, 20% wheat brane, 10% soy bean meal, 14% corn, 5% wheat straw, 0.5% salt and 0.5 limestone. The chemical composition of the basal diet is shown in Table 1. Ewes on the second group T2 (as positive control), fed the basal diet and supplemented with 3g Lysin /animal/day (F-LYS) free lysine (Lysine HCl 85%), while the third group T3 was fed basal diet supplemented with 3g protected lysin /animal/day (P-LYS). The rumen-protected lysine (LysiGEM) in this experiment were obtained from Kemin industries, Belgium. Each ewe was housed in an individual pens (1.5 x 1.5m^2) to ensure its consumption from offered concentrate at the rate of 1000 g daily and wheat straw *ad libitum* in addition to grazing for 6 hr per day, all ewe have free access to clean water. Milk yield was harvested at day 15 from each period. The yield was determined using double oxytocin injection method as shown by Doney et al (14). Lambs were remained with their mothers except for the time when milk yield was recorded. At the day of test, the ewes were separated from their lambs and were injected with 1ml of oxytocin (Oxytocin, Argentina) to stimulate milk let down and milked to evacuate the udder via hand milking. The lambs were reseparated from their dams for 4 hours, and then were re-injected with 1mL of oxytocin and milked again. The milk yield of 24 hours was calculated by multiplying the yield within 4 hours by 6. Milk samples (50ml) from each ewe was analyzed for their composition using an ultrasonic milk analyzer Ekomilk (Eon Trading LLC, USA). Milk energy values were calculated using the following equation: Calorific value (MJ/kg) = 1.64+0.42 × fat% according to Economides, (17). The equation from Mavrogenis and Papachristoforou (28) was used to calculate the fat-corrected milk (FCM): 6% FCM = M (0.453 + 0.091F). Energy-corrected milk (ECM) was determined following the equation of Peterson et al (35) as follows: ECM= (0.327×milk kg/d) + (12.86×Fat Kg/d) +(7.65 ×Protein Kg/d).

Table 1. Chemical composition of concentrate mixture g/Kg DM[1].

DM	CP	OM	Ash	EE	CF	NFE[2]	ME Mj/kg DM[3]
923	148.5	957.2	42.8	45.3	47.8	715.6	13.44

1 Chemical composition[1] AOAC, (4)
2 NFE= OM - (CP + CF+ EE).
3 ME MJ/kg DM = [(CP × 0.012) + (EE × 0.031) + (CF × 0.005) + (NFE × 0.014)] MAFF, (26).

Blood samples from each ewe were collected via jugular vein before morning feeding at the end of each period and centrifuged at 3000 rpm for 20 minutes to harvest blood serum which was stored at -20°C until analysis. Serum total protein (g/dl), albumin (g/dl), triglyceride (mg/dl), cholesterol(mg/dl), glucose mg/dl and urea (mg/dl) levels were determined calorimetrically using analytically commercial kits (Olasmatec Laboratory product, U.K). Globulin content of each serum sample was obtained by subtracting albumin contents from the serum total protein concentration. Rumen liquor was collected from each ewe on day 14 at 0 and 3 h after morning meal via esophageal tube and was filtered through 4 layers of cheese cloth and pH was measured in fresh samples using a portable pH-meter (BP3001), the probe was washed between samplings then samples were frozen at -20^{O}C for later analysis for ammonia concentrations and protozoal count. Ammonia nitrogen was measured according to MAFF (27), 5 ml of rumen fluid was diluted with 45 ml of tungestic acid and kept under -20°C in plastic bottles for further analysis. Rumen protozoa counting was calculated as described by Dehority, (13).

Statistical Analyses: The data were statistically analyzed using SAS (39) software as in the following model:

$$Yij(k) = \mu + Pi + Yj + Tk + \sum ij(k)$$

Where Yij is the observed value of trait;
μ is the overall mean;
Pi is the effect of the row (periods);
Yi is the effect of the column (replicate);
Tk is the effect for treatments,
ij(k) is the value of the experimental error.
Significant differences among means were tested according to Duncan (16).

RESULTS AND DISCUSSION

Dry matter intake (DMI) and milk production: The effects of dietary protected lysine supplementation on concentrate dry matter intake and milk production are presented in Table 2. Concentrate DMI increased significantly for ewes supplemented with P-LYS (895.60 g/d) or F-LYS (885.57 g/d) respectively, compared to control (850.95 g/d). In agreement with our findings, in dairy ewes, Goulas et al (20) reported that during the suckling period the inclusion of animal fat plus 5 g rumen protected methionine resulted in an increase of DMI significantly by 14.7% compared to control. A similar result was noted in cows by other workers (10 ;40).

Table 2. Milk performance, chemical composition, and milk' constituents yield in local ewes fed rumen-protected lysine (P-LYS) and free lysine (F-LYS) supplements.

Item	Overall mean	Control	F-LYS	P -LYS
Concentrate DMI g/d	877.37±5.9	850.95±9.55 b	885.57±3.16 a	895.60±2.86 a
Milk yield gm/day	667.33±3.42	532.22±59.43	670.88±111.06	798.89±137.51
	Milk fat			
Content %	7.51±0.24	7.34±0.41	7.63±0.43	7.55±0.45
Yield gm/day	50.93 ±5.55	40.01±5.66	52.52±10.11	60.26±11.85
	Milk protein			
Content %	6.17±0.08	6.15±0.16	6.17± 0.42	6.21±0.45
Yield gm/day	40.93±3.83	32.87±3.85	41.08±6.63	48.86±8.25
FCM	781.54±81.05	642.0±97.69	781.8±141.40	920.9±172.20
ECM kg/d	1.21±0.12	0.99±0.15	1.43±0.26	1.21±0.22
Milk energy MJ/Kg	5.17±0.10	5.09±0.17	5.18±0.19	5.24±0.18

*** Means with different superscripts within each row differ significantly (p<0.05).**
ECM= (0.327× Milk Kg/d + (12.86×Fat Kg/d) + (7.65 × Protein Kg/day). Peterson (35).
Milk energy MJ/Kg = 1.64+ 0.42 × fat%. Economides, (17).

Daily milk yield averaged 667.333 ± 63.43 gm/day/ewe. The present study revealed that daily milk production of local ewes that supplemented with rumen protected lysine, (LysiGEM) may enhance milk production which increased numerically, (798.9±137.51 g/d/ewe) as compared to T2 F-LYS (670.9±111.06 g/d/ewe) and negative control group (532.2±59.43g/d/ewe). Such increase amounted to 33.4 %, and 16% as compared to control and F-LYS respectively (Table 2). The lack of significant difference in milk yield may be due in part to the fact that the experimental periods were short. From the results of this study, it can be suggested that supplementation the local ewe's diet with 3gm/day/ewe rumen protected lysine may enhance the amount of AAs particularly in the small intestine available for absorption and may have resulted in an improvement of milk production (8; 12). Similarly, a higher milk yield was noticed as compared to control when feeding ewes, a diet with rumen protected methionine and lysine (42), or receiving 0.28% encapsulated lysine plus 0.11 % encapsulated methionine (24). A similar finding has been also reported in sheep (25; 33), in goats (19; 36) and in cows (11; 41; 45).

Milk composition: In the present work, a non-significant (P>0.05) increase in milk fat and yield (g/d) was found in ewe's diet supplemented with P-LYS (7.55±0.45 % and 60.26±11.85 g/d) or F_LYS (7.63±0.43% and 52.52±10.11) as compared to control (7.34±0.41% and 40.01±5.66 g/d). Similarly, in goat, Poljičak-Milas and Marejak (36) noted a non-significant effect on milk fat following rumen protected methionine supplementation. A similar trend was reported in cows by other investigator (23; 46; 48; 53). The overall means of milk protein percentage and yield are 6.17±0.08 % and 40.93±3.83 g/day, respectively. No differences were observed following supplementation of P-LYS or F-LYS to local ewes' diet as compared to control. This result was similar to those reported earlier in lactating goats (30; 36). The dietary supplementation of P-LYS and F-LYS did not affect (P >0.05) the energy corrected milk kg/d (ECM) and fat corrected milk (FCM 6%/kg), which was (1.43±0.26 and 1.21±0.22 kg/d) and (920.9±172.20 and 781.8±141.40 kg/d) for P-LYS and F-LYS as compared to control group (0.99±0.15 kg/d) and 642.0±97.69 kg/d), respectively. In agreement with our findings, Mavrommatis et. al (29) reported that ewes fed rumen-protected lysine did not affect ECM and FCM. Results revealed a non-significant effect of supplemented with P-LYS and F-LYS diets on milk energy which averaged for T1, T2 and control (5.18and 5.24 vs 5.09 MJ/kg, respectively). In contrast to this study, feeding rumen protected protein significantly (P>0.05) increased net energy (NE) content of milk in Awassi ewes (21) and Meriz goats (15).

Blood biochemical: The effect of supplementation of P-LYS and F-LYS to the diet of lactating ewes on serum biochemical parameters are given in Table (3). Neither rumen protected lysine nor free lysine had a significant (P>0.05) effect on total protein, albumin, globulin, triacylglycerols, cholesterol and glucose. Such result is in accordance with those reported by Elwakeel et al (18) in sheep. Also, Younis and Abd-Elazem (54) noted that the effect of supplementation with protected amino acids on plasma biochemical parameters of Barki ewes was not significant. Moreover, in Hariana heifers, Singh et al (44) found no effect of protected methionine and lysine on total protein, albumin and globulin.

Table 3. Serum biochemical parameters in local ewes fed rumen-protected lysine (P-LYS) and free lysine (F-LYS) supplements

Item	Overall mean	Control	F-LYS	P-LYS
Total protein, g/dl	**6.49±0.19**	**6.62±0.32**	**6.63±0.31**	**6.23±0.39**
Albumin, g/dl	**2.89±0.09**	**2.94±0.10**	**2.95±0.17**	**2.78±0.18**
Globulin, g/dl	**3.60±0.15**	**3.67±0.30**	**3.68±0.25**	**3.44±0.27**
Triglycerides, mg/dl	**18.07±1.33**	**17.78±2.42**	**19.33±3.08**	**17.11±1.30**
Cholesterol, mg/dl	**47.88±2.15**	**48.22±4.25**	**50.22±3.90**	**45.23±3.19**
Glucose, mg/dl	**57.22±3.59**	**57.33±8.57**	**56.67±4.55**	**57.67±5.61**
Urea, mg/dl	**34.25±1.52**	**36.66±2.86 a**	**31.11±2.17 b**	**33.00±2.40 b**

*** Means with different superscripts within each rows differ significantly (p<0.05).**

The only blood biochemical parameter which appeared to be affected by the supplementation of protected and free lysine was blood urea, which was appreciably lower ($p<0.05$) in P-LYS (31.11 mg/dl) and F-LYS (33.00 mg/dl) groups as compared to control (36.66 mg/dl). Such findings are in agreement with Younis and Abd-Elazem (54), who noticed that supplementation of both LYS and MET significantly decreased urea N concentration in Barki sheep plasma. The dietary inclusion of RPAA resulted in a significant decline in the urea concentration of dairy sheep blood. Decrease ($P<0.05$) in blood urea nitrogen in the ewes fed rumen-protected lysine and free lysine might represent an improvement in their nitrogen balance (49). Extensive degradation of high-quality protein by microorganisms in the rumen results in some losses of nitrogen as urea in the urine (5). In cows, Wang et al (51), and Sai et al (38) reported a significant reduction in urea concentration in the serum of cows whose diets were supplemented with both methionine and lysine. In contrast, Mustafa, (32) and AL-Dabagh, (2), noted that blood urea concentration was significantly higher ($P<0.01$) in ewes fed protected diets as compared to control.

Rumen parameters: the effect of supplementation P-LYS, F-LYS on rumen fluid parameters are presented in Table 4. Ruminal pH before feeding and after 3h of feeding was not affected by the treatment. In accordance to our results In Bakri sheep, Younis and Abd-Elazem (54), Saanen goats Abbasi et al (1) and in cattle, Tamura et al (47) and Wanapat et al (50). No significant ($P>0.05$) differences were observed on concentration of NH_3-N and protozoa count following supplementation with P-LYS, F-LYS to local ewes. However, ewes fed rumen protected lysine or free lysine groups have a trend to increase ammonia nitrogen concentration (18.98 mg/dl and 18.02 mg/dl), respectively compared to control (16.95 mg/dl). This increment might be due to enhancing protein degradation which led to have a trend to increase NH_3-N concentration (22). Similarly, Robinson et al (37) found that either adding 10 g/kg DM free or protected lysine increased linearly the concentration of NH_3-N in rumen fluid. They hypothesized that feeding ruminant diet contain lysine might enhance protein degradation which led to increase NH_3-N concentration inside rumen fluid (22).

Table 4. Some rumen parameters in local ewes fed rumen-protected lysine (P-LYS) and free lysine (F-LYS) supplements.

Item	Overall mean	Control	F-LYS	P-LYS
pH				
Before feeding	**7.08±0.07**	**7.07±0.10**	**6.99±0.09**	**7.18±0.16**
After feeding 3h	**5.91±0.08**	**5.77±0.10**	**5.99±0.18**	**5.97±0.17**
NH_3-N mg/dl				
Before feeding	**15.9 2±1.57**	**16.44±2.03**	**15.42 ±0.92**	**13.71±0.91**
After feeding 3h	**17.98±0.94**	**16.95±1.74**	**18.02±1.77**	**18.96±1.50**
Protozoa, $\times 10^5$/ml	**125.74±2.99**	**124.11±6.05**	**126.00±6.91**	**127.11±4.51**

*** Mean with different superscripts within each rows differ significantly ($p<0.05$).**

CONCLUSION

Supplementing protected lysine and free lysine resulted in an improvement of milk yield and its composition compared to ewes fed the control rations. Significant ($P<0.05$) decrease was noticed in blood urea nitrogen without any adverse effect on rumen parameters. More research is needed to be carried out by using protected form amino acids lysine-HCl and methionine as additive to determine their effect on production performance of local Iraqi ewes.

ACKNOWLEDGEMENTS: The author would like to thank Professor Dr. Jalal E. Al-Kass for his valuable help in reading the manuscript

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