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Effect of Protected Lysine and Methionine as Feed Additives on the Productive Performance of Some Dairy Animals

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Abstract

The study aimed to assess the impact of essential amino acids, specifically lysine and methionine, on the diets of Holstein dairy cows. Thirty healthy, high-yielding cows aged three to four years were divided into three groups: control, lysine, and lysine plus methionine. The results revealed significant variations in dry matter intake (DMI) among the groups. The total solids (TS) percentage was higher in the lysine plus methionine group (13.63%) and the lysine group (13.46%) compared to the control group. Regarding fat-corrected milk (FCM), the group receiving lysine plus methionine demonstrated the highest increase (43.46), followed by the lysine group (39.36), while the control group had a lower increase (36.81). After calving, the control group exhibited a higher concentration of beta-hydroxybutyrate (BHBA) (1.29 mmol/L), whereas the lowest concentration (0.81 mmol/L) on day 28 was observed in the lysine plus methionine group, followed by the lysine group (1.08 mmol/L). Non-esterified fatty acids (NEFA) concentration decreased in all treatments before and after parturition. The composition of certain fatty acids was not affected, except for a higher level of C15:0 in the lysine plus methionine group (6.81%). Both essential and non-essential amino acids displayed significant differences among the various groups. In conclusion, the different treatment groups exhibited significant variances in DMI, TS%, FCM, BHBA concentration, NEFA concentration, and certain fatty acid composition. Moreover, essential and non-essential amino acids also demonstrated significant differences across the groups.

***Keywords**:* Feed; Additives; Productive Performance; Milk;Lysine; Methionine; Holstein

Introduction

Lysine (Lys) and methionine (Met) have traditionally been recognized as the most limiting amino acids (AA) for lactating dairy cows fed corn-based diets **(Schwab *et al*., 1992; NRC, 2001)**.Protein nutrition of dairy cows in recent years has shifted from the use of dietary crude protein (CP) towards meeting the ammonia and AA needs for ruminal fermentation in order to maximize microbial protein (MCP) synthesis **(Schwab and Broderick, 2017).**Another nutritional shift seen recently is the balancing of individual AA in metabolizable protein (MP) as a method to deliver bioavailable AAs to the small intestine. However, in order for an AA product to reach the small intestine for absorption, it must be protected from rumen microbial degradation. Protection of free AAs from ruminal degradation dates to the 1960s **(Schwab and Broderick, 2017)** and numerous rumen-protected AA (RPAA) products, have been developed using a variety of technologies. In terms of Met, encapsulation of a Met molecule with coating materials, such as carbohydrates or polymer allows RPAA products to avoid ruminal

degradation **(Schwab and Ordway, 2003).** Altering the structural configuration of AAs to avoid microbial degradation is another method to escape rumen degradation. Methionine analogues utilize a hydroxyl group to protect them from ruminal degradation and are converted to useable Met by enzymatic reactions **(Schwab and Ordway, 2003).** Rumen-protected Lys (RPLys) products are often coated by a series of lipid or fatty acid calcium-based salts **(Ji *et al*., 2016).** Encapsulated rumen protected Met (RPMet) and Met analogues can impact milk yield, but their effects tend to be more prominent in altering milk fat and protein composition **(Patton, 2010; Zanton*et al*., 2014).** Encapsulated RPLys supplementation has minimal impact on production parameters, unless RPLys is supplemented with a RPMet product **(Robinson, 2010).** Lactational responses to both 2 supplemental RPMet and RPLys in the literature are inconsistent and require further research. Inconsistent findings may be related to AA protection methods, varying supplementation rates, or metabolizable protein concentrations in the diets. The N-acetyl-L-Met (NALM), ƐN-acetyl-L-Lys (εNALL), and Nα, ε-acetyl-L-Lys (diNALL) are AA derivatives and developmental forms of RPAAs for lactating dairy cows. Based on prior research, it is thought that the acetyl group acts as a barrier to block the hydrolysis of the AA N-terminal **(Wallace, 1992).** As a result, the goal of this study is to evaluate the impact of essential amino acids (lysine and methionine) on the diets of Holstein dairy cows.

Material and methods

Experimental Animals, Feeding and Management

Experimental Design and Treatments

Animal Experimentation Ethics Committee approved all experimental procedures (protocol 2021), Aswan University. This study carried on 30 healthy, high-yielding Holstein dairy cows between the ages of three and four-year-old. Based on the number of lactations, previous lactation yield, and body condition score before the close-up, three study groups with ten cows each were created: the control group (Con), the lysine group (Lys), the lysine plus methionine group (lys+Met).

The cows in each group were fed a basal diet. The cows in lysine treatment (Lys), were enhanced with 30 g of rumen protected lysine (RPL), while lys+Met group enriched with 20 grams of rumen protected methionine (RPM) plus 30g RPL, all supplementation of rumen protected lysine, methionine to the cows in the treatment groups were given in the amounts according to the NRC requirements and the bioavailability recorded by manufacture company. From 21 pre-calving to 30 days in milk, all animals received the same close-up ration and the same lactation ration. The ration's ingredients and chemical composition were determined in accordance with **NRC (2001)** recommendations.

Animal Management and Feed

Between November 2021 and June 2022, experimental studies were carried out in a private Animal Production farm, El-Gharbia governorate, Egypt. The cows that were used in this study were kept in a free-standing open stall. As previously indicated **(Cetin *et al*., 2018),** close-up and lactation rations were mixed daily and fed as a total mixed ration. After experimental period, the animals returned to the farm herds. During the trial, health issues like metritis, a displaced abomasum, a retained placenta, milk fever, and ketosis were recorded.

Rations chemical analysis (dry matter, crude protein, ether extract, ash, calcium, and phosphorus) were in accordance with **AOAC (2010)** procedures and determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF).

Experimental materials Rumen protected Lysine, LysiGEM^{TM}, kemin company, this product has 70% lysine HCL, 50 % lysine, and provide 444 g lysine/kg of product. So, the amount to be added in the ration will be 11.3 x 1000/444 = 25.45=30 g.

Rumen protected methionine, KESSENT®, kemin company, this product has 75 % DL Methionine, with bioavailability >80 %, provide 606g methionine /kg of product.

So, the amount to be added in the ration will be 10.3x1000/606= 16.99≈20 g.

Sampling and Biochemical Analysis of Blood

Coccygeal vein using vacuum tubes for serum after feeding (4 hours later) at wk 3 after calving, to determine BHBA. Serum samples were collected after centrifugation of the blood at 3,000 x g for 15 min. Plasma and serum were stored at -20 C until analysis.

Chemical analysis of milk samples

The samples were mixed and analyzed in duplicate for moisture, total solids (TSS), fat, protein, total acidity (as lactic acid) and pH. Protein content was determined by using the micro-Kjeldahl method and was obtained by multiplying the percentage of total nitrogen (TN) by 6.38 for milk ingredients and 6.25 for plant ingredients, fat content was measured by the Gerber method **(Kurt *et al*., 1996)** and TS were determined using a drying oven **(AOAC, 2010).** Titratable acidity was expressed in terms of % lactic acid **(Anonymous, 1989).** pH was determined by electric pH meter (HANNA HI 2211). Total carbohydrates were calculated by difference.

Fatty acid methyl esters preparation:

The fatty acid composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 1.0mL of n-hexane to 15 mg of oil followed by 1.0mL of sodium methoxide (0.4 mol), according to the modified method of **Zahran and Tawfeuk (2019).**

Fat corrected milk and Energy corrected milk, feed efficiency

Milk determines the amount of milk produced adjusted to 3.5% butterfat and 3.2% protein.

The suggested equation of FCM 3.5% fat = 0.35 Milk per Kg + 18.57 Fat kg, where this formula is valid and should be used in place of FCM (4%) **(Parekh, 1986).**

Energy Corrected Milk is used to determine the energy amount in milk, where it is based upon milk amount, butter fat and milk protein and adjusted to 3.5% fat and 3.2% protein, also [2]ECM were estimated as mentioned plus milk lactose= 0.01 milk + 12.2 fat + 7.7 p + 5.3 L.

The cow's ability to convert dry matter feed into milk pounds. It is the ratio of pounds of milk produced per pound of dry matter feed. Feed efficiency was calculated by dividing the 3.5%FCM yield (kg/d) by DM intake (kg/d).

Blood Biochemical Analysis

Plasma total proteins (g/dl) were determined according to the method described by **Henry (1974),** The determination of plasma albumin (g/dl) based on a colorimetric method described by **Dumas *et al.* (1997)**. Globulin was calculated by subtracting plasma albumin from plasma total protein and then A/G ratio was calculated.

Plasma total lipids (mg/L) were determined according to the method of **Knight *et al.* (1972).** Cholesterol (Total, LDL and HDL) was determined according to the method of **Richmond (1973).** Triglycerides (mg/dl) was determined by the method of **Stein and Myers (1995),** Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and

creatinine levels were spectrophotometrically determined by using available commercial kits as described by the manufacturer companies (Spectrum, Diagnostics, Egypt. Co. for Biotechnology, S. A. E). Procedure for colorimetric estimation of non-esterified fatty acids (NEFA) was standardized using the extraction method of **Itaya and Ui (1965).**

Statistical analysis

Tests and analyses including each sample and each test parameter mentioned above were conducted in triplicate. The results are reported as the mean ± SD. The collected data were statistically analyzed using the general linear model in IBM SPSS Statistics 25 software, and the Duncan's multiple range test was applied to determine significance at a p-value of ≤0.05.

Results and discussion

Table 1 represents the ration composition per head per day for livestock, prepared according to the NRC (National Research Council) guidelines of 2021. The table includes various ingredients and their respective amounts on a dry matter (DM) basis and an as-fed basis. The total amount of the ration is 24.385 kg on dry matter basis and 41.485 kg on fed basis.

Table (1): The ration was prepared according to NRC, 2001.

Ration composition per head per day		
Ingredients	**Amount per kg**	
	On DM	**AS-fed**
Egyptian lucerne hay	4	4..62
Corn silage	7.82	23
Corn grain, fine grounded	3.35	3.8
Soyabean meal	2.5	2.8
Distillers' grains with soluble	3.6	4.04
Dry fat, fractionated 99%	0.3	0.3
Corn gluten feed	6	6.73
Vegetable oil	0.05	0.05
Urea	0.2	0.2
Limestone	0.2	0.2
Sodium bicarbonate	0.3	0.3
Salt	0.03	0.03
Min- vit. Premix	0.035	0.035
Total	24.385	41.485

***** Each 3 kg Min and vit premix contains: Vit A 4800000 IU; Vit D3 1000000 IU; Vit E 28000 mg; Zinc 100000 mg; Manganese 80000 mg; Iron 75000 mg; Copper 30000 mg; Iodine 750 mg; Cobalt 200 mg; Selenium 300 mg

Table (2) appears to be an analysis of a ration prepared for a fresh period for cows producing 40 kg of milk. The table includes various parameters related to the nutritional composition of the ration and the corresponding supply, demand, and balance values. Metabolizable energy: The predicted supply of metabolizable energy is 277 MJ, while the demand is 281 MJ. This indicates a slight energy deficit (-4 MJ) in the ration. NDF (Neutral Detergent Fiber): The NDF content in the dry matter (DM) of the ration is 30.5%, and the predicted supply of NDF is 7.01 kg. The eNDF (%NDF) is 72.4, suggesting a moderately good supply of effective NDF for proper rumen function. Starch and Sugar: The starch content in the DM is 26.8%, and the sugar content is 5.1%. These values contribute to the energy content of the ration. NFC (Non-Fiber Carbohydrates): The predicted supply of NFC is 40.7%. NFC includes sugars, starch, and other readily fermentable carbohydrates. It provides a source of energy for the cow. Metabolizable protein: The predicted supply of metabolizable protein is 2635 g, with a balance of 193 g, indicating an excess of protein in the ration.RDP/UDP protein:

The RDP (Rumen Degradable Protein) and UDP (Undegradable Dietary Protein) percentages are given as 61.2% and 38.8%, respectively. These values relate to the different protein fractions in the ration. The predicted supply of calcium is 184.7 g, with a balance of 8 g. The predicted supply of magnesium is 51.1 g, with a balance of 1.9 g. These minerals are important for various physiological processes in cows. DCAD (Dietary Cation-Anion Difference): The calculated DCAD is 327 meq/kg. These results were in agreement with **(NRC, 2001; Weiss, 2008)**. It is a measure of the balance between cations (positively charged ions) and anions (negatively charged ions) in the ration and can affect cow health and milk production.

Table (2): The predicted analysis of ration prepared for fresh period to cows produced 40 kg milk

Metabolizable energy		NDF (%DM)	30.5	Starch (%DM)	26.8
Supply (MJ)	277	NDF (kg)	7.01	Sugar	5.1
Demand	281	eNDF (%NDF)	72.4	NFC	40.7
Balance	-4	NDF frg (%NDF)	71	Forage: conc.	48:52
Metabolizable protein		RDP/UDP protein		ASH (%DM) 5.3	
Supply (g)	2635	RDP (%CP)	61.2		
Demand	2443	UDP(%CP)	38.8		
Balance	193				
Calcium (g)		Magnesium (g)		DCAD	
Supply (g)	184.7	Supply (g)	51.1	Calculated	327
Demand	176.6	Demand	49.9	Recommended	>250
Balance	8	Balance	1.9		
Methionine		Lysine		Choline	
% MP	6.22	1.78			
Demand Metabolizable(g)	178.3	Metabolizable(g)	59.3	Demand (g)	>12.9g choline in late gestation and early lactation
Supply	168	Supply	48	Totally degraded in rumen	
Balance	-10.3	Balance	-11.3		

Table (3) provided shows the impact of supplementation of rumen-protected lysine and methionine on dry matter intake (DMI), milk production, and composition in lactating dairy cows, both before and after calving. The control sample represents the cows that did not receive any supplementation of rumen-protected lysine or methionine. Lysine + methionine: This group received supplementation of both rumen-protected lysine and methionine. Lysine: This group received supplementation of rumen-protected lysine only. The table presents the average values for each parameter, along with their standard deviations. The values are given separately for the pre-partum and post-partum periods (specifically, 30 days after calving). Pre-Partum: Control: The DMI was 9.3±0.57 kg, milk production was 38±0.3 liters per day, lactose content was 1.65±0.0037%, milk fat content was 1.26±0.029%, and milk protein content was 1.66±0.03%. Lysine + Methionine: The DMI was 10.5±0.11 kg, milk production was 42.23±0.18 liters per day, lactose content was 2.28±0.012%, milk fat content was 1.54±0.007%, and milk protein content was 1.77±0.04%. Post-Partum (30 days after calving): Control: The DMI increased to 18.46±0.27 kg, milk production increased to 42.23±0.18 liters per day, lactose content remained similar at 1.65±0.0037%, milk fat content increased slightly to 1.26±0.029%, and milk protein

content increased slightly to 1.66±0.03%. Lysine + methionine: The DMI increased to 20.53±0.06 kg, milk production increased to 42.23±0.18 liters per day, lactose content increased to 2.28±0.012%, milk fat content increased to 1.54±0.007%, and milk protein content increased to 1.77±0.04%. This result is agreement with **(Reynolds *et al*., 2003; Huhtanen and Hristov, 2009).** Comparing the treatments, it can be observed that supplementation with both lysine and methionine resulted in higher DMI, milk production, and improved milk composition compared to the control group. Supplementation with lysine alone also showed some improvements but to a lesser extent.

Table (3): The impact of supplementation of Rumen protected lysine and methionine on DMI, milk production and its composition on the lactating dairy cow, pre- and post-partum

Treatments	Pre-partum DMI	Post-partum (30 days) DMI	Milk per liter	Lactose	Milk fat	Milk protein
Control	9.3±0.57d	18.46±0.27c	38±0.3d	1.65±0.0037a	1.26±0.029a	1.66±0.03a
Lysine+ methionine	10.5±0.11c	20.53±0.06b	42.23±0.18ab	2.28±0.012c	1.54±0.007c	1.77±0.04b
Lysine	10.33±0.88ab	20.13±0.20b	41.1± 0.20c	2.22±0.009b	1.34±0.005b	1.74±0.02ab

Table (4) provides information on the impact of supplementation of rumen-protected lysine and methionine on the body condition score (BCS) of dairy cows at 30 days post-partum. The BCS is a numerical rating system that indicates the amount of body fat and energy reserves in cows, with higher scores generally indicating better body condition. The treatments mentioned in the Table are as follows: Control treatment: This group represents cows that did not receive any supplementation of rumen-protected lysine or methionine. Lysine + methionine treatment: This group received supplementation of both rumen-protected lysine and Methionine. Lysine treatment: This group received supplementation of rumen-protected lysine only. The Table displays the BCS values for each treatment, both pre-partum and at 30 days post-partum. The values are given as means with their respective standard deviations. These results are agreement with **Roche *et al.* (2009).** From the Table, it can be observed that supplementation with both lysine and methionine resulted in a higher BCS at 30 days post-partum compared to the control group. Supplementation with lysine alone also showed some improvement in BCS, but to a lesser extent than the combination of lysine and methionine.

Table (4): The impact of supplementation of Rumen protected lysine and methionine on BCS of dairy cow at 30-day post-partum

Treatments	**BCS**	
	Pre-partum	**Post-partum (30 days)**
Control	4±0.00	2.50 ± 0.14^{c}
Lysine+ methionine	4±0.00	3.00 ± 0.14^{b}
Lysine	4±0.00	2.83 ± 0.08^{ab}

Table (5) shows that the chemical composition of cow milk affected by different formulas of feeding with lysine and methionine. The control treatment has a TS% of 12.43%, lysine treatment has 13.44%, and lysine+ methionine treatment has 13.63%. The highest TS% is observed in the lysine+ methionine treatment. The Control treatment has a protein% of 4.36%, Lysine treatment has 4.31%, and lysine+ methionine treatment has 4.12%. The lowest protein% is observed in the lysine+ methionine treatment. The control treatment has a fat content of 3.30%, Lysine treatment has 3.27%, and lysine+ methionine treatment has 3.65%. The highest fat content is observed in the lysine+ methionine treatment. These results are agreement with **Smith *et al*. (2018).** The control treatment has a lactose content of 4.13%, lysine treatment has 5.21%, and lysine+ methionine treatment has 5.21%. The highest lactose content is observed in

both the lysine and lysine+ methionine treatments. The control treatment has an acidity% of 0.16%, lysine treatment has 0.16%, and lysine+ methionine treatment has 0.15%. The lowest acidity% is observed in the lysine+ methionine treatment. The pH values are consistent across all treatments, with no significant differences observed. The control treatment has an SNF% of 6.62%, lysine treatment has 6.62%, and lysine+ methionine treatment has 6.65%. The SNF% values are similar across all treatments. Ash%: The Control treatment has an ash% of 0.636±0.003a, Lysine treatment has 0.65%, and lysine+ methionine treatment has 0.65%. The ash content is similar across all treatments. From the results, it appears that the lysine+ methionine treatment generally exhibits higher TS, fat, and lactose (%) compared to the other treatments. However, it has a lower protein content compared to the control and lysine treatments. The acidity (%) is lower in the lysine+ methionine treatment. The pH, SNF%, and ash% values do not show significant differences among the treatments. These results are agreement with those of **(Garcia-Bojaca *et al.*, 2019; Yang (2020).**

Table (5): The impact of supplementation of rumen protected lysine and methionine on chemical milk composition, acidity (%) and pH values

Treatments	**TS%**	**Protein %**	**Fat %**	**Lactose %**
Control	12.43±0.06^{c}	4.36±0.1^{a}	3.30±0.05^{b}	4.13±0.01^{b}
Lysine	13.44± 0.13^{b}	4.31±0.12^{b}	3.27±0.008^{c}	5.21±0.003^{a}
Lysine+ methionine	13.63± 0.06^{a}	4.12±0.08^{c}	3.65±0.003^{a}	5.21±0.005^{a}
Treatments	**Acidity %**	**pH**	**SNF %**	**Ash %**
Control	0.16±0.00^{a}	6.62±0.00^{a}	9.13±0.012^{c}	0.636±0.003^{a}
Lysine	0.16±0.00^{a}	6.62±0.00^{a}	10.17±0.12^{a}	0.65±0.003^{aa}
Lysine+ methionine	0.15±0.00^{b}	6.65±0.00^{a}	9.98±0.07^{b}	0.65±0.01^{a}

* Means (three different determinations) ± standard deviation (SD)

Table (6): The impact of supplementation of rumen protected AA on Fat corrected milk; energy corrected milk and feed efficiency

Treatment	**FCM**	**ECM1**	**ECM2**	**Feed efficiency**
Control	36.81±0.61^{d}	41.6±0.2^{e}	37.4±0.13^{e}	1.99±0.017^{b}
Lysine+ methionine	43.46±0.2^{b}	44.43±0.21^{d}	44.79±0.09^{c}	2.11±0.016^{a}
Lysine	39.36±0.17^{c}	47.13±0.08^{c}	42.28±0.23^{d}	1.95±0.011

* Means (three different determinations) ± standard deviation (SD)

Based on the provided results in Table (6), it appears that different treatments had varying effects on the parameters measured, including FCM (Feed Conversion Ratio), ECM1, ECM2, and feed efficiency. Here's a brief comment on the results: The control group (Con) demonstrated a feed conversion ratio (FCM) of 36.81, while treatment with lysine and methionine resulted in an improved FCM of 43.46, indicating enhanced feed efficiency. Similarly, lysine supplementation alone led to a further increase in FCM to 39.36. In terms of ECM1 and ECM2, the lysine and methionine treatment showed values of 41.6and 37.4, respectively, while lysine supplementation alone resulted in higher values of 47.13and 42.28for ECM1 and ECM2, respectively. This suggests that both treatments had positive effects on extracellular matrix production. These results suggest that lysine and methionine

supplementation, as well as lysine supplementation alone, had beneficial effects on various parameters, including FCM, ECM1, ECM2, and feed efficiency.

Table (7) shows that, in the pre-partum period, all treatments showed similar values for milk yield, with the control group and both lysine and methionine treatments exhibiting milk yields of 0.15±0.003a. However, during the post-partum period, some variations were observed. In the control group, milk yield gradually increased from 0.16±0.02ab on day 14 to 0.99 at day 0 (parturition). It then stabilized around 0.89 to 0.92 until day 14 and slightly decreased to 0.72 and 0.71 at days 21 and 28, respectively. In summary, the control group exhibited a gradual increase in milk yield during the pre-partum period, followed by a relatively stable phase post-partum. Similar finding was observed by **(Sova *et al.*, 2018; Santos, 2020).** The lysine and methionine treatment showed a similar trend but with slightly lower milk yields, while the lysine treatment resulted in a different pattern with varying milk yields.

Table (7): The impact of supplementation of rumen protected AA. on NEFA (mmol/L)

	Pre –partum (days)				
Treatment	**-21**	**-14**	**-7**		
Control	0.15±0.003^{a}	0.16±0.02^{ab}	0.28±0.017^{a}		
Lysine +methionine	0.15±0.003^{a}	0.18±0.005^{a}	0.24±0.005^{b}		
Lysine	015±0.003^{a}	0.12±0.003^{c}	0.21±0.008^{b}		
	Post-partum (days)				
Treatment	**0**	**7**	**14**	**21**	**28**
Control	0.99±0.04^{a}	0.89±0.04^{a}	0.92±0.044^{a}	0.72±0.004^{a}	0.71±0.00^{a}
Lysine +methionine	0.81±0.005^{b}	0.74±0.005^{b}	0.74±0.005^{b}	0.63±0.017^{b}	0.6±0.006^{b}
Lysine	0.76±0.005^{bc}	0.62±0.003^{c}	0.66±0.01^{bc}	0.54±0.03^{c}	0.48±0.015^{c}

Table (8): The impact of supplementation of rumen protected AA. on lipids profile (mg/dl).

lipids profile	**Control**	**Lysine+ methionine**	**Lysine**
VLDL	6.1±0.05^{e}	7.5±0.15^{c}	7±0.04^{d}
LDL	76.96±0.98^{a}	28.86±0.37^{d}	34.53±0.31^{c}
HDL	75.66±0.66^{c}	88.66±1.2^{a}	84.56±1.37^{ab}
Triglyceride	45.43±0.29^{a}	33.53±0.29^{d}	34.51±1.26^{c}
Cholesterol	178±31.77^{b}	171.5±9.5^{c}	118.5±2.5^{e}

Table 8 shows that the impact of supplementation of rumen protected amino acids (AA) on the lipids profile measured in milligrams per deciliter (mg/dl). It is important to note that the letter annotations indicate significant differences between the groups. Overall, the supplementation of lysine+methionine and lysine showed positive effects on the lipids profile by reducing LDL and triglyceride levels and increasing HDL levels. These results are in agreement with **Naseem *et al.* (2018).**

Table 9 shows that the impact of supplementation of rumen protected amino acids (AA) on the protein profile. It is important to note that the letter annotations indicate significant differences between the groups. Overall, the supplementation of lysine+methionine and lysine showed positive effects on the protein profile by increasing total protein and albumin levels, without significant changes in globulin. Furthermore, both supplemented groups exhibited lower urea levels, indicating potential improvements in nitrogen metabolism. These results are in agreement with those of **(Mousa *et al*., 2017; Lin., 2018).**

Table (9): The impact of supplementation of rumen protected AA on protein profile

protein profile	Control	Lysine+ methionine	Lysine
total protein (g/dl)	7.39±0.02^{b}	7.71±0.02^{a}	7.67±0.08^{a}
Albumin (g/dl)	2.97±0.003^{c}	3.08±0.003^{b}	3.06±0.01^{b}
Globulin	4.41±0.02^{a}	4.62±0.02^{a}	4.61±0.09^{a}
Urea (mg/dl)	62.85±1.47^{a}	45.63±0.28^{d}	47.51±0.76^{cb}
Creatinine (mg/dl)	1.24±0.02^{b}	1.22±0.01^{b}	1.31±0.005^{a}

Conclusion

The study aimed to assess the effects of lysine and methionine supplementation on dry matter intake (DMI), total solids (TS%) content, fat-corrected milk (FCM) levels, beta-hydroxybutyrate (BHBA) concentration, non-esterified fatty acid (NEFA) concentration, and fatty acid composition in comparison to a control group. The study involved thirty healthy, high-yielding cows divided into control, lysine, and lysine plus methionine groups. The results demonstrated significant variations in DMI, TS%, FCM levels, BHBA concentration, NEFA concentration, and specific fatty acid composition among the treatment groups, indicating the impact of essential amino acid supplementation on these parameters.

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