Turkish Journal of Veterinary & Animal Sciences

Manuscript 4357

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**Turkish Journal of Veterinary and Animal Sciences**

**http://journals.tubitak.gov.tr/veterinary/**

**Research Article**

Turk J Vet Anim Sci
(2024) 48: 207-217

doi:10.55730/1300-0128.4357

# Effects of supplementing rumen-protected methionine and lysine on milk yield and milk composition in dairy cows

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**Received:** 31.03.2024 • **Accepted/Published Online:** 08.09.2024 • **Final Version:** 09.10.2024

**Abstract:** The use of health-food additives has gained significant attention within the dairy sector, with a focus on enhancing the nutritional intake of dairy cows to improve their productivity and reproductive performance. This study aims at examining the impact of rumen-protected methionine (Met) and lysine (Lys) supplementation on production and reproductive parameters in a sample of 100 dairy cows, comprising 32 primiparous and 68 multiparous cows with an average age of 5.24 ± 2.31 years. The cows' average milk production was determined to be 24.9 ± 7.40 kg/day. The cows were divided into two groups, one receiving Met/Lys supplementation and the other serving as a control group. The supplemented group demonstrated superior performance in various metrics: milk yield (25.61 kg/day vs. 24.16 kg/day, $p < 0.0001$), fat (979.02 g/day vs. 874.27 g/day, $p = 0.08$), protein (843.98 g/day vs. 761.50 g/day, $p = 0.10$), and lactose content (4.36% vs. 4.21%, $p = 0.003$). Supplementation also led to elevated levels of butyrate and protein. Notably, the supplemented cows exhibited higher body condition scores (BCS) at different stages of lactation, with BCS values of 3.01 at 35 days and 2.80 at 65 days, compared to 2.83 and 2.61 in the control group. Additionally, Met/Lys supplementation resulted in higher levels of albumin (33.22 g/L) and urea (0.20 g/L) in serum samples, with a tendency towards increased levels of aspartate aminotransferase (51.20 U/L). Reproductive performance showed improvements in the supplemented group with a lower number of services per conception (1.88 vs. 2.05), a higher conception rate (44.83% vs. 22.22%), and a higher total pregnancy rate (55% vs. 48%), although these differences were not statistically significant. Thus, the addition of rumen-protected Met and Lys to dairy cow rations shows potential for enhancing milk production and composition, as well as improving specific metabolic and reproductive characteristics.

**Key words:** Rumen-protected methionine, rumen-protected lysine, milk production, milk composition, reproductive performance

## 1. Introduction

The dairy industry's primary goal is to maximize dairy cow productivity and health. Advances in animal nutrition, particularly protein nutrition [1–3], play a critical role in improving dairy herd performance, particularly through the use of feed supplements. Among these additives, the supplementation of essential amino acids, such as methionine and lysine, has gained popularity [1,2]. When these amino acids are protected from the rumen, they can be absorbed directly into the small intestine, increasing their availability for essential metabolic processes [4,5]. This study investigates the effects of rumen-protected methionine and lysine supplementation on milk production and composition in dairy cows.

Methionine (Met) and lysine (Lys) are two essential amino acids that dairy cows cannot produce in sufficient quantities to meet their physiological requirements. As a result, these amino acids must be obtained through diet alone. Rumen microbes degrade a large portion of dietary protein in the rumen, resulting in a limited supply of essential amino acids for intestinal absorption. Protecting these amino acids from rumen degradation ensures that a greater proportion arrive intact in the small intestine, where they can be absorbed and used by the body [6–8].

Met is essential for protein synthesis, antioxidant production, and lipid metabolism. It also helps prevent hepatic steatosis, which is common in high-producing dairy cows [5,7]. Lys is necessary for tissue growth and repair, collagen production, and immune function. Together, these amino acids have the potential to significantly improve dairy cow health and productivity. Previous research [1,2] has shown that rumen-protected

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Met and Lys can improve milk production, milk composition, and body condition in dairy cows. Cows receiving these supplements, for example, produced more milk and had higher levels of milk fat and protein. These gains are attributed to improved nutrient utilization and reduced metabolic stress, which allows cows to convert feed into milk more efficiently.

Furthermore, rumen-protected Met and Lys can help dairy cows reproduce successfully [1,2]. Improved nutritional balance can result in higher conception rates and shorter calving intervals. This is especially important on dairy farms, where profitability is dependent on the cows' ability to maintain consistent, productive lactation cycles. The effect of rumen-protected Met and Lys supplementation on cow metabolic health is also a promising area of study. Optimal levels of these amino acids can help regulate energy metabolism and lower the risk of metabolic disorders like ketosis and hepatic steatosis. Furthermore, improved metabolic health can lead to lower veterinary costs and longer cow lifespans, helping dairy farms to remain sustainable.

## 2. Materials and methods

### 2.1. Animals and farm

The study of rumen-protected amino acid supplementation (Met + Lys) was conducted on a grazing dairy farm located in North Central Algiers (36°42′N, 3°13′E) and North Eastern Setif (36°09′N, 5°26′E) using data collected from a follow-up of two dairy cattle farms from mid-October 2019 to mid-July 2021. The study included 100 imported dairy cows (43 Montbéliardes, 37 Normandes, and 20 Prim'Holsteins) with an average age of 5.24 ± 2.31 years. There were 32 primiparous and 68 multiparous cows producing 24.9 ± 7.40 kg of milk per day. All dairy cows were in early lactation, grouped according to their expected calving date, and matched by breed, parity, and milk quantity from the previous lactation. They were randomly divided into two groups to ensure proper application of the experimental protocol during each calving period. Cows in stanchion barns were milked twice daily, at 4 AM and 4 PM, with milk production recorded daily throughout the experiment.

### 2.2. Diets

During the experimental period, the daily feed ration for experiment A consisted of 1 feeding × 2 kg of alfalfa hay, 4 × 4 kg of concentrate 1, 4 × 2 kg of corn silage, and 3 × 3.5 kg of concentrate 2, supplemented by 2 × 2.5 kg of corn grain and 5 h of green feed and pasture. Straw was distributed ad libitum (4–5 kg per cow per day), and cows had free access to water and mineral blocks at all times.

Concentrate 1 in experiment A is a mixture of complete feeds with varying protein and fiber contents. This feeding concept is based on North American practice and was originally adapted for soilless systems. The mixture contains a variety of feeds, including dry forages (approximately 11% long-strand alfalfa type and 5% straw), raw materials, and additives (approximately 36% corn grain, 15% barley, and 0.7% urea). Concentrate 2 in experiment B is composed of corn, soybean meal, milling waste (wheat bran), and barley grain.

Table 1 shows an approximate analysis of the feed ration [9]. Calculations of dry matter (DM), crude protein (CP, Kdjeldhal method), fat (Soxhlet method), mineral matter (MM), neutral detergent fiber (NDF, Van Soest neutral detergent treatment), acid detergent fiber (ADF, Van Soest acid detergent treatment), acid detergent lignin

**Table 1.** Average nutritional composition[1] (% dry matter) of the total mixed ration offered to cows in the two experiments[2,3] from day 0 to day 85.

| | Feed | | | | | Concentrate | |
|---|---|---|---|---|---|---|---|
| | Straw[2,3] | Wheat hay[3] | Alfalfa hay[2] | Maize silage[2] | Concentrate 1[2] | Concentrate 2[3] | Maize[3] |
| DM, %. | 90.35 | 92.00 | 89.70 | 28.71 | 99.80 | 90.63 | 78.32 |
| CP, %. | 8.87 | 5.43 | 30.99 | 24.07 | 41.81 | 13.32 | 9.79 |
| Fat, % MS | 1.03 | 0.98 | 1.13 | 7.73 | 2.61 | 8.89 | 5.76 |
| MM, % MS | 9.06 | 6.45 | 9.88 | 22.50 | 4.96 | 9.10 | 2.30 |
| CC, % MS | 40.12 | 44.09 | 32.89 | 74.64 | 4.39 | 11.09 | 2.10 |
| ADF, % MS | 53.44 | 44.24 | 36.41 | 25.51 | 13.81 | 6.69 | 4.69 |
| NDF, % MS | 82.43 | 69.02 | 49.40 | 45.43 | 52.39 | 22.84 | 12.90 |
| ADL, % MS | 8.91 | 6.85 | 8.44 | 22.19 | 3.90 | 2.35 | 1.02 |
| HC, % DM | 32.74 | 24.02 | 32.82 | 19.92 | 38.58 | 17.82 | 70.61 |
| C, % DM | 40.8 | 35.05 | 48.49 | 3.32 | 9.91 | 4.78 | 26.23 |
| L, % MS | 18.37 | 15.88 | 21.52 | 19.09 | 2.89 | 1.38 | 0.22 |

**Table 1. (Continued)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Met/Lys product, g/cow/day | | | | | | | |
| DL-methionine [2, 3] | - | - | - | - | 12 | 12 | - |
| L-lysine HCl[2, 3] | - | - | - | - | 30 | 30 | - |

[1]Samples of forages and concentrates used by dairy cows were taken monthly between day 0 and day 85 to calculate dry matter (DM), crude protein (CP, Kdjeldhal method), fat (Soxhlet method), mineral matter (MM), neutral detergent fiber (NDF, Van Soest neutral detergent treatment), acid detergent fiber (ADF, Van Soest acid detergent treatment), acid detergent lignin (ADL, Van Soest 72% sulfuric acid treatment), and at the feed laboratory of the Superior National Veterinary School of Algiers, Algeria. The quantities of the organic matter fractions hemicellulose (HC), cellulose (C), and lignin (L) are the result of successive extractions by highly chemically aggressive reagents (NDF, ADF, and ADL) eliminating by successive subtractions the residual organic matter at the end of each extraction stage.
[2]Ration for experiment A: straw, alfalfa hay, corn silage, concentrate 1 (complete feed with highly variable composition: corn grain, soybean meal, grain barley, alfalfa hay, cane molasses, bicarbonate, urea, rock salt, straw, kernel).
[3]Ration for experiment B: straw, wheat hay, corn, concentrate 2 (corn, soybean meal, wheat bran milling waste, and barley grain).

(ADL, Van Soest 72% sulfuric acid treatment), and crude cellulose (Weende method) were carried out at the feed laboratory at the Superior National Veterinary School of Algiers, Algeria.

From the day of calving to 85 days postpartum, cows in the Met/Lys supplemented group each received 12 g of rumen-protected DL-methionine (DL-methionine Rhodimet NP99, Adisseo, France) and 30 g of rumen-protected L-lysine HCl (L-lysine monohydrochloride 99% feed grade, Ajinomoto, France), as recommended by NRC [8], while cows in the control group received their conventional ration without any supplement. The Met/Lys incorporated additive was presented in 25 kg bags as a dry white-to-cream powder before being supplied with the concentrates in the form of compressed cores having a physical coating encapsulating the "nus" amino acids in a barrier layer composed of hydrophobic high-melting point lipids [10]. This technique is one of the new granulation technologies recently applied in Algeria to protect amino acids from contact with the rumen's liquid phase to ensure the prevention of methionine and lysine decomposition by rumen microbiota. This formulation of coated methionine and lysine, which the cow can assimilate, represents a potential source of dietary protein synthesis precursors (99% DL-methionine, 78% L-lysine HCl), which reduces the need to increase dietary protein, increases the availability of these protected nutrients, and improves nitrogen utilization efficiency [7,11]. According to Rulquin et al. [10], these two coated amino acids will be released after digestion of the lipid layer by enzymes in the small intestine.

### 2.3. Data collection and sample analysis

Each cow's average milk production was recorded on days 15, 30, 45, 60, and 75. Individual milk samples (0.5 to 1 L) were taken on days 15, 35, and 65 for qualitative analysis (fat content, protein content, and lactose) using an analyzer (Lactostar, Funke Gerber 3510). Individual blood samples were collected from the tail vein on postpartum days 7, 14, and 21 to determine plasma levels of various biochemical parameters. Blood was collected in heparinized tubes 4 h after ingestion of the morning ration when ruminal fermentation is at its peak and end products are released following supplementation [12]. The blood samples were centrifuged at 3000 rpm for 15 min at 4 °C. The serum was collected, identified, and stored at –20 °C before being delivered to the biochemical analysis lab. Serum concentrations of glucose, triglycerides, cholesterol, albumin, total protein, urea, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) were measured using an integrated analyzer (Kenza 240 TX-Biolabo Group, Les Hautes Rives, Maizy-France) modified for blood analysis with commercial colorimetric kits. The interassay coefficients of variability of the glucose, triglycerides, cholesterol, albumin, total protein, urea, ALAT, and ASAT were 13.66%, 29.22%, 28%, 12.57%, 11.26%, 29.67%, 14.17%, and 13.89%, respectively. Insemination was performed after the cows exhibited estrus, based on daily observation of behavioral signs by the farm personnel. Rectal palpation was used routinely to confirm the pregnancy diagnostic after the last insemination by the veterinary practitioner. All data related to the management of cow reproduction were recorded using methods designed for that purpose, and were systematically monitored. Reproductive performance was estimated on the basis of the four intervals of calving to first heat, calving to first service, calving to conception, and first service to conception, and then by calculating the six parameters of first service conception rate, services per conception, conception rate, fertility index, apparent gestation rate, and total gestation rate.

### 2.4. Statistical analyses

Multivariate statistical analysis was carried out in a randomized manner using SAS v.9.3 [13]. The effects of ruminal degradation-protected methionine and lysine supplementation on milk quantity and quality in dairy cows and the serum levels of various biochemical parameters were measured over time using the SAS GLM procedure. The SAS FREQ procedure was used to assess

the effect of methionine and lysine supplementation on body condition scores (BCS) while protecting against ruminal degradation.

The nonparametric Mann–Whitney test was used to estimate the effect of ruminal degradation-protected methionine and lysine supplementation on dairy cow reproduction, as the distribution of values does not follow a normal distribution. The interaction between supplementation and study day was analyzed for all variables except the reproductive parameters, for which the study day was not considered. A descriptive analysis of the collected data was performed first on the milk production and milk composition variables, followed by the BCS and biochemical profile, and finally on the reproduction variables. Significant treatment differences were defined as $p \leq 0.05$, and trends as $0.05 < p \leq 0.10$.

## 3. Results

In each experiment, adding coated DL-methionine and L-lysine HCl increased milk yield significantly over the 12-week lactation period. The increase was most noticeable in the first 2 weeks of lactation (+1.12 kg/day, Figure 1). However, there were no significant changes in milk composition (butyrate and protein content) or the biochemical composition of the blood (Figure 2). The significant effect of supplementation over time was recorded only for lactose ($p = 0.003$, Figure 3). Supplementation had a highly significant effect ($p < 0.004$) on all productive performance parameters studied (milk yield, fat, and lactose content), except for butyrate and protein content, which tended to increase with the addition of encapsulated methionine and lysine ($p = 0.08$ and $p = 0.10$, respectively).

Figure 4 shows no significant effect ($p = 0.21$) of supplementation over time on BCS. On day 7, supplementation had no significant effect on cow's body condition ($p = 0.22$); however, on day 35 and day 65, Met/Lys intake had a significant influence on BCS compared to the control group, respectively by 3.01 vs. 2.83 on day 35 ($p < 0.02$) and 2.80 vs. 2.61 on day 65 ($p < 0.003$) (Figure 4). Cows in the supplemented group showed smaller variations in BCS ($\Delta BCS \approx 0.22$) compared to cows in the

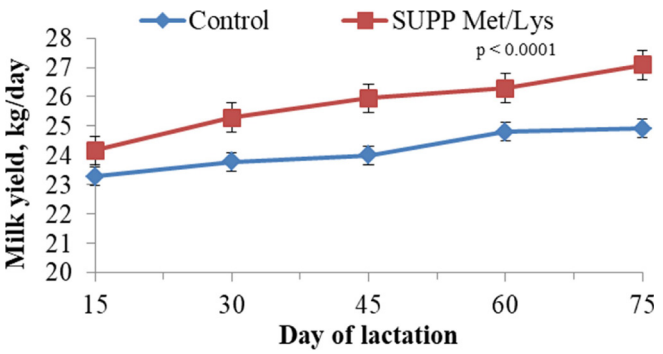


**Figure 1.** Milk yield of dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85. *The error bars represent the standard error of the mean.*

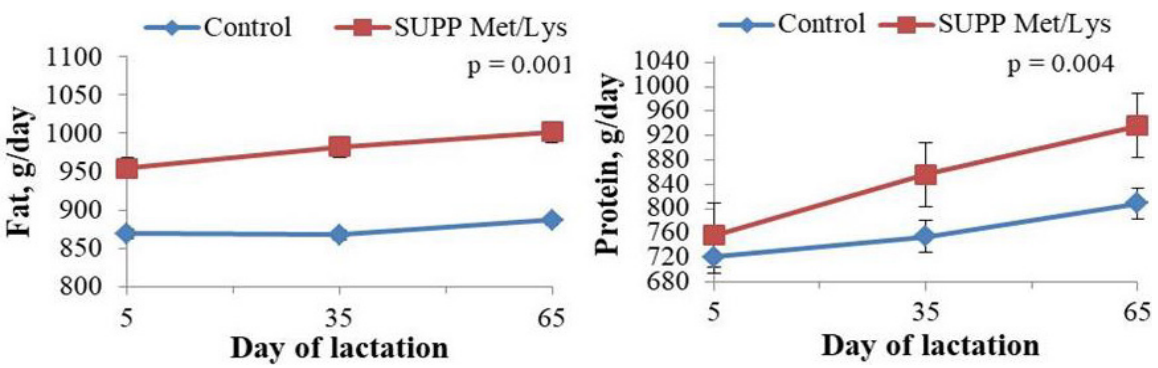


**Figure 2.** Fat and protein (g/day) in the milk of dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85. *The error bars represent the standard error of the mean.*

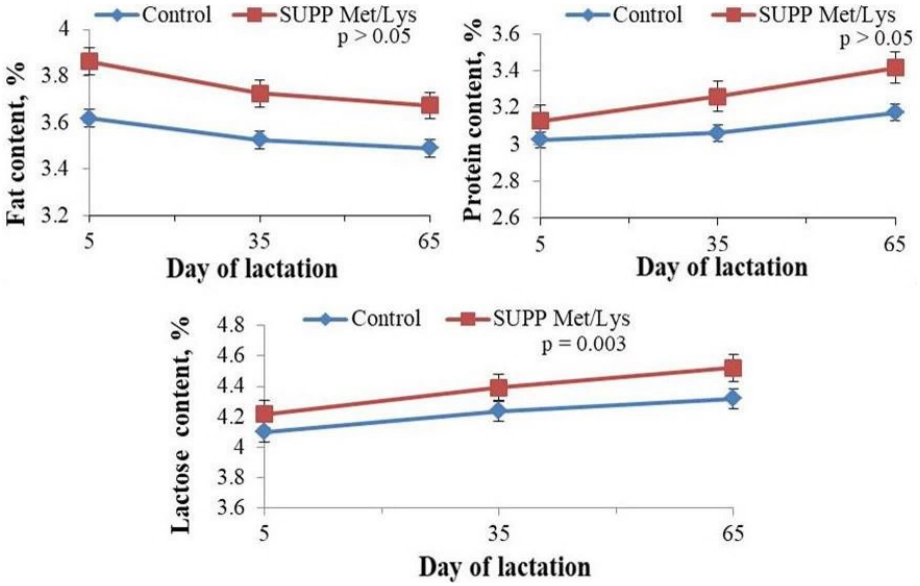


**Figure 3.** Fat, protein and lactose content (%) in the milk of dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85. *The error bars represent the standard error of the mean.*

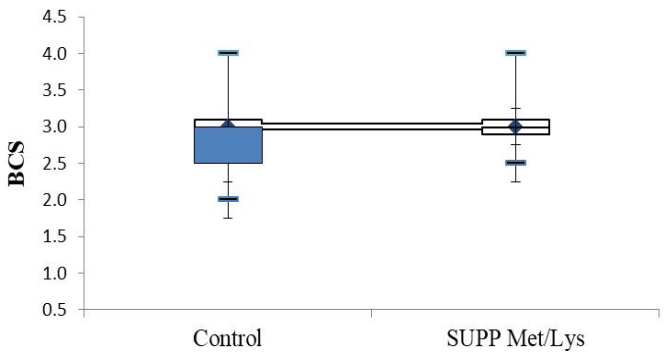


**Figure 4.** Box plot showing body condition change (BCS) of dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85.

control group (ΔBCS ≈ 0.26). With regard to biochemical parameters, a significant effect of supplementation over time was recorded for triglycerides ($p = 0.05$, Table 2). Similarly, albumin and urea concentrations increased significantly after Met/Lys supplementation ($p < 0.03$, Table 2).

Plasma concentrations of the three energy parameters glycemia, cholesterolemia, and triglyceridemia followed similar trends in both groups, ranging from 0.32 to 0.79 g/L for glucose, 0.31 to 2.04 g/L for cholesterol, and 0.04 to 0.39 g/L for triglycerides. During the first 3 weeks, blood glucose, cholesterol, and triglycerides increased insignificantly ($p > 0.05$) by +0.08, +0.32, and +0.06 g/L, respectively. However, both groups showed a significant increase at day 21.

In terms of nitrogen parameters, albumin and uremia were significantly different between the two groups, at 32.53 vs. 33.79 g/L ($p = 0.03$) and 0.18 vs. 0.21 g/L ($p = 0.002$), respectively. No significant variation in plasma total protein concentration was observed during the study ($p = 0.52$), with the exception of total protein, which showed a remarkable increase (73.81 g/L) in the Met/Lys-supplemented cows by day 21.

Regarding reproductive performance (Table 3), cows supplemented with digestible Met and Lys had slightly

**Table 2.** Plasma concentrations of glucose, triglycerides, cholesterol, albumin, total protein, urea, ALAT and ASAT in dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85. *SEM represents the standard error of the mean.*

| | Supplementation Met/Lys | | SEM | p-value | |
|---|---|---|---|---|---|
| | Control group | Supplemented group | | Supp. | Supp. × day |
| Glucose, g/L | | | | | |
| D7 | 0.48 | 0.53 | 0.07 | | 0.58 |
| D14 | 0.51 | 0.56 | 0.07 | 0.25 | |
| D21 | 0.53 | 0.60 | 0.08 | | |
| $P^2$ | <0.0001 | <0.0001 | | | |
| Triglycerides, g/L | | | | | |
| D7 | 0.18 | 0.17 | 0.057 | | |
| D14 | 0.19 | 0.20 | 0.058 | 0.99 | 0.05 |
| D21 | 0.21 | 0.23 | 0.06 | | |
| $P^2$ | <0.0001 | <0.0001 | | | |
| Cholesterol, g/L | | | | | |
| D7 | 1.08 | 1.17 | 0.35 | | |
| D14 | 1.11 | 1.19 | 0.34 | 0.96 | 0.43 |
| D21 | 1.23 | 1.22 | 0.28 | | |
| $P^2$ | <0.0001 | <0.0001 | | | |
| Albumin, g/L | | | | | |
| D7 | 32.26 | 32.61 | 4.43 | | |
| D14 | 32.74 | 33.87 | 3.88 | 0.03 | 0.25 |
| D21 | 32.58 | 34.89 | 4.41 | | |
| $P^2$ | =1.0000 | =1.0000 | | | |
| Total protein, g/L | | | | | |
| D7 | 67.10 | 67.66 | 8.99 | | |
| D14 | 68.62 | 70.08 | 7.72 | 0.52 | 0.17 |
| D21 | 69.29 | 73.81 | 7.14 | | |
| $P^2$ | =1.0000 | =1.0000 | | | |
| Urea, g/L | | | | | |
| D7 | 0.18 | 0.19 | 0.05 | | |
| D14 | 0.18 | 0.22 | 0.06 | 0.002 | 0.55 |
| D21 | 0.19 | 0.22 | 0.07 | | |
| $P^2$ | <0.0001 | <0.0001 | | | |
| ALAT, U/L | | | | | |
| D7 | 32.13 | 33.16 | 4.78 | | |
| D14 | 32.96 | 34.65 | 4.51 | 0.92 | 0.75 |
| D21 | 33.69 | 35.73 | 5.23 | | |
| $P^2$ | =1.0000 | =1.0000 | | | |
| ASAT, U/L | | | | | |
| D7 | 49.20 | 50.82 | 8.36 | | |
| D14 | 50.64 | 51.98 | 6.77 | 0.07 | 0.57 |
| D21 | 52.33 | 51.98 | 6.01 | | |
| $P^2$ | <0.0001 | <0.0001 | | | |

higher fecundity parameters ($p \geq 0.25$) compared to the control cows, with averages of 76.10 vs. 83.90 days, 131.86 vs. 143.96 days and 54.22 vs. 55.31 days for the calving to conception interval (days open), calving to first service interval (waiting period), and first service to conception interval, respectively. Similarly, fertility parameters showed service per conception values of 1.88 vs. 2.05 and first service conception rates of 44.83% vs. 22.22% for the supplemented and control groups, respectively.

## 4. Discussion

In recent years, there has been a focus on the concept of 'precision nutrition', which has resulted in the development of more detailed tools and technologies based on enriching the dairy cow diet with essential nutrients. Proteins and their amino acid building blocks are among these nutrients. Most notably, metabolizable Met and Lys account for 7.5% and 2.4%, respectively, according to NRC [8] recommendations. In theory, protein intake for dairy cattle could be reduced by providing amino acid supplements that are resistant to ruminal degradation, thereby increasing nitrogen utilization in cows and consequently increasing the volume and constituents of milk produced [11,14]. The magnitude of the increase in milk volume observed during these experiments (+1.45 kg/day) is negligible when compared to the increases reported by Zhou et al. [14] and Batistel et al. [15] of 4.1 kg/day and 4.4 kg/day, respectively, during the high production period (31–60 days in milk) of cows fed digestible Met. Milk yield increases throughout lactation due to a sufficient supply of digestible Met or Lys, which is primarily explained by resistance to ruminal degradation [16,17].

Similar to the current study, early lactation experiments in dairy cows and ewes show a significant increase ($p < 0.05$) in milk production (+1.45 kg/day), either with or without a significant positive effect on protein content [18]. However, the significant increase of more than 1.12 kg/day in the average quantity of milk produced during the first 2 weeks of lactation in cows receiving digestible Met/Lys corroborates the results obtained by Rémond [19,20], who reported that the supply of protected DL-methionine increased the quantity of milk produced during the first and second weeks by a highly significant increase equal to 2.6 kg/day ($p < 0.01$) and 1.2 kg/day ($p < 0.05$), respectively. Some other studies [20] have evaluated the effects of rumen-protected Met or Lys supplementation during various stages of lactation.

Our findings are similar to those of Zhao [21], who discovered that cosupplementing with rumen-protected Met, threonine, isoleucine, and leucine on a low-protein diet (12% crude protein) significantly improved milk yield and lactose content ($p < 0.05$) while milk protein yield remained unchanged ($p \geq 0.05$). On the other hand, the modest improvement in milk volume observed in the current study contradicts the findings of some authors, who demonstrated that nitrogen feed correction using protected amino acids has no effect on milk yield [22–24]. According to Fagundes et al. [25], supplementation with N-acetyl-L-Met has no effect on milk yield. The lack of change in milk volume is completely consistent with the

**Table 3.** Reproductive performance of dairy cows fed a basic diet with (supplemented group) or without (control group) enrichment in DL-methionine and L-lysine HCl protected against ruminal degradation (12 g/cow/day of DL-methionine + 30 g/cow/day of L-lysine HCl) from day 0 to day 85. *SEM represents the standard error of the mean.*

| | Supplementation Met/Lys | | SEM | p-value |
|---|---|---|---|---|
| | Control group | Supplemented Group | | Supp |
| Number of cows for breeding | 41 | 41 | - | - |
| Number of fertilized cows | 29 | 36 | - | - |
| Number of cows inseminated ≥ 3 times | 13 | 8 | - | - |
| Calving to the first heat interval, days | 40.96 | 37.35 | 12.18 | 0.30 |
| Calving to first service interval, days | 83.90 | 76.10 | 30.66 | 0.25 |
| Calving to conception Interval, days | 143.96 | 131.86 | 71.60 | 0.50 |
| First service to conception interval, days | 55.31 | 54.22 | 68.98 | 0.95 |
| First service conception rate, %. | 29.27 | 39.02 | - | 0.14 |
| Services per conception | 2.05 | 1.88 | 1.02 | 0.45 |
| Conception rate, % | 44.83 | 22.22 | - | 0.27 |
| Apparent Fertility Index | 2.93 | 2.47 | - | - |
| Total Fertility Index | 2.07 | 1.83 | - | - |
| Apparent gestation rate, % | 34 | 40 | - | - |
| Total gestation rate, % | 48 | 55 | - | - |

findings of feeding experiments involving increasing amounts of distillers' grains in diets with or without rumen-protected Lys supplementation on milk yield, composition, and plasma amino acid concentration [26]. Supplementation with absorbable amino acids (Met and Lys) significantly increased milk fat (+104.75 g/day, $p = 0.001$) and true protein content (+82.48 g/day, $p = 0.004$), as previously reported by some authors [15,16,27].

Furthermore, the current study's significant lack of improvement in fat and protein concentrations ($p = 0.10$ and $p = 0.24$, respectively) is consistent with the findings of Broderick et al. [28] and Phipps et al. [29]. Our findings on the cows' lack of response to supplementation with metabolizable Met or an analog are consistent with those reported by Broderick et al. [23]. In contrast to other studies, the authors refuted the absence of response to this supplementation [30,31]. These findings support previous research that found an increase in milk fat [30] and protein [24,31] concentrations in response to metabolizable Met supplementation, with other authors reporting increases in milk fat [32] and protein yields [33].

In the experiments, Met/Lys supplementation slightly increased postpartum BCS in cows in early lactation. This increase was significant from day 35 to day 65 ($p = 0.02$; $p = 0.003$, respectively), with cows compensating for BCS losses during this period by supplementing with undegraded protein and, consequently, energy [34]. Regarding the effect of protected amino acid (Met and Lys) supplementation on the metabolic state of dairy cows, the results of this study show that the plasma concentration of the various biochemical parameters analyzed was slightly increased and without significant variation throughout the study period ($p > 0.07$), with the exception of albuminemia and uremia, which were significantly different.

The albumin levels of the supplemented cows were significantly higher ($p = 0.03$) than those of the control cows. This finding could be attributed to the high level of degradable nitrogen provided by the digestible amino acid supplementation of Met and Lys. Indeed, the primary cause of variation in albumin concentration, which is synthesized by the liver from proteins absorbed in the intestine and proteins from muscle tissue, is the difference between dietary intake and body sampling [35]. In this study, uremia in the control cows was significantly lower ($p = 0.002$) than in the Met/Lys cows. Previous studies, on the other hand, found no significant positive effect on albuminemia or uremia but did find that dietary intake of undegraded amino acids decreased their concentration in the plasma [17,36]. In contrast, adding undegraded Met and Lys increased the concentration of glucose, triglycerides, cholesterol, total protein, and ALAT in cow plasma insignificantly ($p > 0.07$) [17,36]. Met/Lys supplementation tended to increase plasma ASAT concentration ($p = 0.07$). The blood glucose levels reported in the current study (+0.03 g/L) and the non-significant difference in mean values ($p = 0.25$) are similar to those reported in many studies using limiting amino acids protected from ruminal degradation or infused into the digestive tract after the rumen [15,17,36]. Blood glucose levels were slightly higher in the treated cows (0.56 g/L) than in the control cows (0.51 g/L). Triglyceride concentration (0.19 g/L) was not different between the control and the Met/Lys cows, which is consistent with previous research in which early-lactation cows were supplemented with calcium salts of the methionine hydroxyl analog [36].

It is important to note that serum total protein levels were numerically higher in the cows fed Met/Lys supplementation compared to the control cows (70.52 vs. 68.34 g/L), which can be explained by the richness of the base ratio in degraded proteins and the increased nitrogen consumption caused by supplementation with undegraded Met and Lys [17]. Fahey et al. [36] found no significant difference in total plasma protein concentration in cows supplemented with calcium salts of methionine hydroxy analog (76.31 vs. 75.83 g/L). In this study, it can be noted that in addition to significantly improving milk, plasma, and liver production, the addition of coated Met and Lys improved fecundity parameters (calving to first heat interval, calving to first service interval, calving to conception interval, and first service to conception interval), as well as fertility parameters (gestation rate, first service conception rate, and fertility index). However, the improvement remained insignificant ($p > 0.05$).

Several researchers have reported an important role for protein nutrition, specifically the protected amino acids Met and Lys, in improving ovarian activity, egg quality, and fertility rates [37,38], as well as in embryonic growth and survival [39–41] and in the abundance of nutrient transporters in placental tissue [7,42,43]. Similarly, Westwood et al. [44] demonstrated the effects of protected Met on fertility and fertility parameters, reporting that cows fed rumen-degradable protein-rich diets and losing more weight early in lactation were less likely to conceive at first service with a relatively high number of open days.

Hugentobler et al. [45] and Groebner et al. [42] found that physiologically and during gestation, most amino acid concentrations, such as Met and Lys, are higher in the uterus and oviduct than in the plasma. Similarly, recent research [34,36,46] reported results that correspond to our findings regarding reproductive response in which dairy cows fed a diet supplemented with Met and/or Lys protected against ruminal degradation improved their reproductive performance via a net reduction in the average interval between calving and conception, the number of services per conception, and an increase in pregnancy rates. Other studies, however, found that protected amino acid supplementation (Met/Lys) had no effect on dairy cow reproductive response [38].

Supplementing with rumen-protected Met and Lys represents a promising nutritional strategy to enhance milk

production and milk composition in dairy cows. Potential benefits include increased milk production, improved milk quality, enhanced body condition, and positive effects on reproduction and metabolic health. Further research in this field will help to better understand the underlying mechanisms and optimize supplementation protocols to maximize benefits for the dairy industry.

**Informed consent**

All necessary ethical protocols and informed consent procedures were diligently followed throughout the course of this study.

**Acknowledgments**

The authors thank the Setif cooperative farm school "COOPSEL" (Setif, Algeria) for their funding support and their donation of DL-methionine and L-lysine HCl. The authors also thank the Research Laboratory SPA "Animal Health and Production" (Algiers, Algeria) for their funding support.

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