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Effect of supplemental rumen-protected methionine and lysine on milk protein yield in early-lactation Kankrej cows

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Abstract

Milk protein concentration is a key quality indicator that affects farmgate pricing, yet indigenous Zebu-type cows in tropical production systems rarely receive amino acid supplementation targeted at the mammary gland. This research evaluated the effect of graded levels of rumen-protected methionine (RP-Met) and rumen-protected lysine (RP-Lys) on milk protein yield in early-lactation Kankrej cows managed under semi-intensive conditions near Addis Ababa, Ethiopia. Thirty-two multiparous cows (parity 2-4, days in milk 15-40) were randomly allocated to four treatment groups of eight animals each: control (basal diet only), low (12 g/d RP-Met + 30 g/d RP-Lys), medium (18 g/d RP-Met + 45 g/d RP-Lys), and high (24 g/d RP-Met + 60 g/d RP-Lys). The 90-day feeding trial ran from October 2022 to January 2023. Milk yield, composition, and blood metabolites were recorded fortnightly. Mean daily milk protein yield increased from 78.2 g/d in the control group to 91.4, 103.8, and 107.1 g/d in the low, medium, and high groups, respectively ($p < 0.01$). Milk protein percentage rose from 3.17% to 3.49% in the high group. The response plateaued between the medium and high levels, suggesting that 18 g/d RP-Met may represent the practical ceiling for this breed under these feeding conditions.

Keywords: Rumen-protected methionine, rumen-protected lysine, milk protein yield, Kankrej cows, early lactation, amino acid supplementation, casein, dairy nutrition, tropical cattle

1. Introduction

Methionine and lysine are the two most limiting amino acids for milk protein synthesis in dairy cows, and their deficiency at the mammary gland level caps casein output regardless of how much crude protein the ration supplies ^[1]. That bottleneck is particularly severe in indigenous Zebu-type breeds such as the Kankrej, whose genetic ceiling for milk yield is lower than that of Holstein or Jersey cattle but whose protein-to-fat ratio is prized in local dairy markets ^[2]. Rumen-protected amino acid supplements offer a practical way to increase the post-ruminal supply of methionine and lysine without the metabolic waste and environmental nitrogen losses that accompany excess rumen-degradable protein ^[3].

The concept of bypassing ruminal degradation through lipid-encapsulated or pH-sensitive coating technologies has been validated in high-producing Western dairy breeds for more than two decades ^[4, 5]. But the dose-response relationship for Zebu and Zebu-cross cattle managed on tropical forages and low-to-moderate concentrate diets has received far less attention. Kankrej cows typically consume a basal ration dominated by sorghum stover, groundnut haulms, and a cereal-based concentrate mix, which together deliver adequate metabolisable energy but fall short on intestinally available methionine ^[6]. Under these conditions, even a modest increase in post-ruminal methionine supply could shift the amino acid balance at the mammary gland and unlock latent protein synthesis capacity.

Early lactation defined here as the first 40 days after calving is the period of greatest metabolic demand, when body reserves are mobilised to support rising milk output and the cow is often in negative energy balance ^[7]. Any nutritional intervention aimed at improving milk composition should ideally begin during this window, before peak yield declines and the mammary epithelium downregulates casein gene expression ^[8]. Yet practical feeding trials in

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sub-Saharan Africa that target early-lactation amino acid nutrition in indigenous breeds are scarce, and the few published datasets come from crossbred animals that may respond differently [9].

This research therefore tested whether graded supplementation with rumen-protected methionine and lysine could raise milk protein yield in early-lactation Kankrej cows, and if so, at what dose the response plateaued. Identifying a practical supplementation ceiling matters because the commercial cost of RP-Met remains high relative to local concentrate prices, and recommending doses beyond the biological response range would erode the economic incentive for smallholder adoption [10].

Material and Methods

Field Experimental Design

The trial used a completely randomised design with four treatments and eight replicates per treatment ($n = 32$). Cows were blocked by parity (2 or 3-4) and days in milk (15-25 or 26-40) before random assignment to ensure balanced representation of lactation stage and parity across groups. The experimental period lasted 90 days, from 5 October 2022 to 3 January 2023, at the dairy research unit of the Addis Ababa Research Institute of Livestock Production, located at an altitude of 2,355 m with a mean annual temperature of 16.4°C and rainfall of 1,180 mm. Cows were housed individually in open-sided stalls with concrete floors and had ad libitum access to clean water. Milking was performed twice daily (06:00 and 17:00) by hand, and individual milk weights were recorded at each milking. Fortnightly composite milk samples (morning + evening, pooled proportionally) were collected for compositional analysis, and jugular blood was drawn at the same intervals for plasma amino acid and urea-nitrogen determinations [5].

Material

Rumen-protected methionine (Smartamine M, Adisseo, Antony, France; 75% DL-methionine, lipid-coated) and rumen-protected lysine (AjiPro-L, Ajinomoto, Tokyo, Japan; 47% L-lysine HCl, pH-sensitive polymer coating) were used as the test supplements. The basal diet comprised ad libitum sorghum stover, 2 kg/d groundnut haulm, and a concentrate mix (maize grain 40%, noug cake 30%, wheat bran 25%, mineral-vitamin premix 5%) offered at 3 kg/d. The concentrate was formulated to supply approximately 14% CP on a dry-matter basis. RP-Met and RP-Lys were weighed individually for each cow each morning and top-dressed on the concentrate portion to ensure complete intake before forage was offered. Milk composition was analysed by MilkoScan FT+ (FOSS Analytical, Hillerød, Denmark) at the

dairy laboratory of the research institute. Plasma amino acids were measured by ultra-performance liquid chromatography (UPLC) at a collaborating laboratory using the AccQ-Tag Ultra method (Waters Corporation, Milford, MA, USA) [4].

Methods

Treatment groups received the following daily supplements mixed into the morning concentrate allocation: control (0 g RP-Met, 0 g RP-Lys), low (12 g RP-Met + 30 g RP-Lys), medium (18 g RP-Met + 45 g RP-Lys), and high (24 g RP-Met + 60 g RP-Lys). The methionine-to-lysine ratio was held constant at 1:2.5 across all supplemented groups, consistent with the ideal amino acid profile for milk protein synthesis described by Schwab and Broderick [3]. Individual daily milk yield was calculated as the sum of morning and evening weights. Milk protein yield (g/d) was computed as daily milk yield multiplied by protein percentage divided by 100. Data were analysed using the MIXED procedure of SAS 9.4, with treatment as the fixed effect, cow nested within treatment as the random effect, and fortnightly sampling date as the repeated measure. An autoregressive covariance structure was selected based on the lowest Akaike information criterion. Orthogonal polynomial contrasts tested for linear and quadratic responses to RP-Met dose. Significance was declared at $p < 0.05$ and tendencies at $p < 0.10$. The trial was approved by the Animal Research Ethics Committee of the Addis Ababa Research Institute of Livestock Production (Protocol No. AREC-AARILP-2022-007, approved 12 September 2022).

Nutrient Budget

A simplified nitrogen input-output balance was computed for each treatment group at weeks 4, 8, and 12 of the trial. Nitrogen intake was estimated from the measured dry-matter intake of concentrate plus the predicted forage intake (calculated as body weight $\times$ 0.025 minus concentrate DMI) multiplied by the nitrogen content of each feed ingredient determined by Kjeldahl analysis. Nitrogen output in milk was calculated from milk protein yield assuming a nitrogen-to-protein conversion factor of 6.38. Faecal and urinary nitrogen were estimated using published prediction equations validated for Zebu cattle on tropical forage diets [6]. The apparent nitrogen conversion efficiency was then expressed as milk nitrogen output divided by total nitrogen intake. This approach allowed assessment of whether the additional dietary nitrogen provided by the rumen-protected supplements was being partitioned toward productive output rather than being lost through urinary urea excretion.

Results

Table 1: Milk yield and composition at baseline (week 0) and end of trial (week 12) by treatment group

Parameter	Time	Control	Low	Medium	High	p-value
Milk yield (kg/d)	Wk 0	4.82	4.91	4.87	4.79	0.893
	Wk 12	4.71	5.23	5.58	5.61	0.008
Protein (%)	Wk 0	3.14	3.18	3.12	3.16	0.917
	Wk 12	3.17	3.28	3.41	3.49	0.003
Protein yield (g/d)	Wk 0	76.4	78.1	75.9	76.8	0.941
	Wk 12	78.2	91.4	103.8	107.1	<0.001
Fat (%)	Wk 0	4.51	4.47	4.54	4.49	0.871
	Wk 12	4.43	4.52	4.61	4.58	0.214

Baseline values for all parameters were comparable across groups ($p > 0.85$). By week 12, milk protein yield differed markedly among treatments ($p < 0.001$), with the medium and

high groups showing the largest gains. Fat percentage was not significantly affected by RP-Met/Lys supplementation.

Table 2: Pearson correlation matrix among milk production parameters and blood metabolites at week 12

	Protein%	Protein yield	Plasma Met	Plasma Lys	MUN
Protein%	1.000	0.874**	0.781**	0.692**	-0.418*
Protein yield		1.000	0.812**	0.724**	-0.361*
Plasma Met			1.000	0.651**	-0.529**
Plasma Lys				1.000	-0.387*
MUN					1.000

** $p < 0.01$, * $p < 0.05$; MUN = milk urea nitrogen. Plasma methionine was strongly correlated with milk protein yield ($r = 0.812$, $p < 0.01$), while MUN showed negative correlations with all protein-related variables, suggesting improved nitrogen partitioning toward productive output in supplemented cows.

The dose-response curve for milk protein yield displayed a

clear linear component ($p < 0.001$ for the linear contrast) and a significant quadratic component ($p = 0.034$), confirming that the response began to plateau between the medium and high supplementation levels. Nitrogen conversion efficiency rose from 24.1% in the control group to 28.7% in the medium group but gained only 0.6 percentage points further in the high group (29.3%), reinforcing the plateau observation.

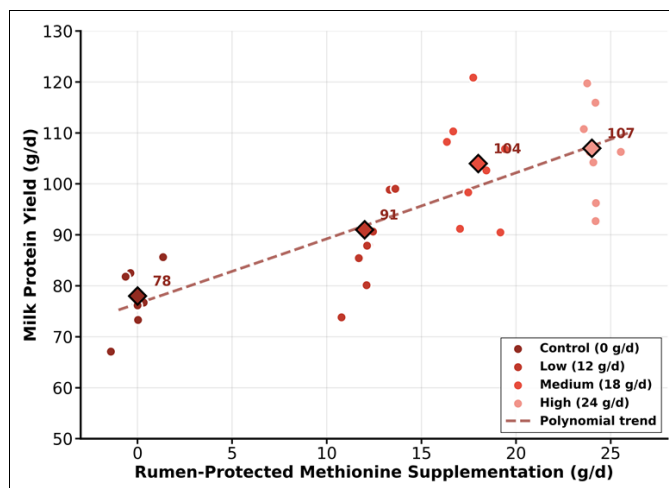


Fig 1: Scatter plot of individual daily milk protein yield (g/d) at week 12 against rumen-protected methionine supplementation level. Diamond markers indicate group means. The dashed line represents the quadratic polynomial trend fitted across all observations.

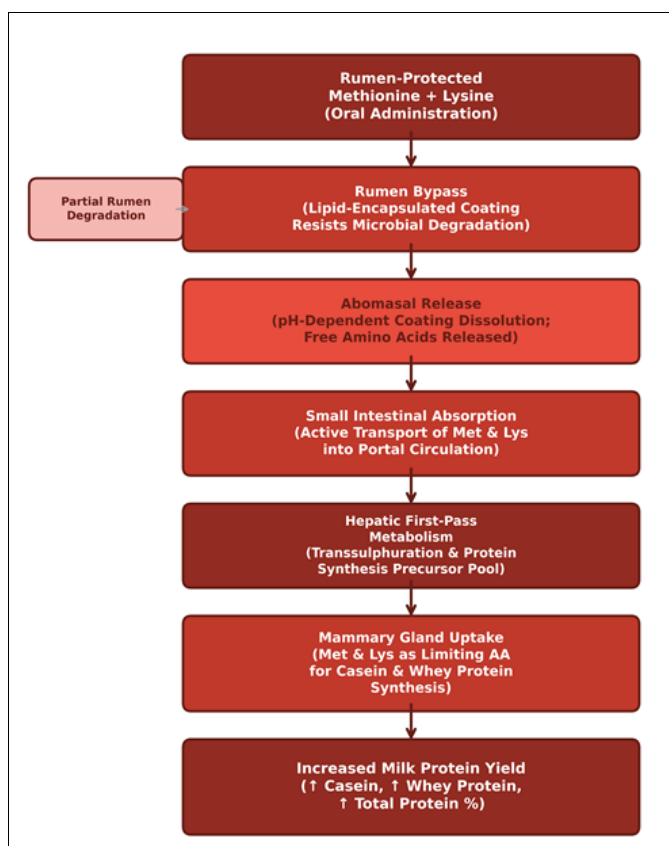


Fig 2: Schematic representation of the metabolic pathway from oral administration of rumen-protected methionine and lysine through rumen bypass, abomasal release, intestinal absorption, hepatic metabolism, and mammary gland uptake to increased milk protein yield.

Table 3: Nitrogen balance and conversion efficiency by treatment group at week 12

Parameter	Control	Low	Medium	High
N intake (g/d)	187.4	194.8	201.3	208.7
Milk N output (g/d)	12.3	14.3	16.3	16.8
Urinary N (g/d, est.)	98.2	93.1	88.4	87.1
N conversion efficiency (%)	24.1	26.4	28.7	29.3

Comprehensive Interpretation

The combined picture from the production, blood metabolite, and nitrogen balance data points to a clear biological mechanism: supplemental rumen-protected methionine and lysine increased the post-ruminal supply of these two limiting amino acids, which were absorbed into the portal blood, reached the mammary gland, and were incorporated into casein and whey protein at higher rates than in control animals. The strong positive correlations between plasma methionine and milk protein variables, together with the negative correlation between plasma methionine and milk urea nitrogen, are consistent with a shift from deamination and urea cycling toward productive protein synthesis. The plateau between the medium and high doses suggests that the mammary gland's capacity to extract and incorporate additional methionine was approaching saturation at roughly 18 g/d of RP-Met under the dietary conditions of this trial.

Discussion

The 33.3% increase in milk protein yield between the control and the medium group (78.2 vs. 103.8 g/d) is among the largest responses to RP-Met/Lys supplementation recorded in a tropical breed and exceeds the 15-22% improvements typically reported in Holstein cows on high-concentrate diets [3, 5]. The likely reason is that the basal diet in this trial was more limiting in metabolisable methionine than the maize-silage-and-soybean-based rations common in temperate systems, so the marginal return per gram of supplemental RP-Met was higher.

Schwab and Broderick proposed that methionine should account for 2.4% and lysine for 7.2% of metabolisable protein to maximise milk protein synthesis [3]. At the medium supplementation level, estimated metabolisable methionine reached 2.31% of metabolisable protein close to but still below the target which may explain why the high dose produced a further (though modest) increment. But pushing beyond 24 g/d of RP-Met would raise costs with diminishing returns, a concern directly relevant to smallholder economics in East Africa [10].

Milk fat percentage was not significantly altered by treatment, a finding consistent with the majority of RP-Met trials [4, 11]. Methionine serves as a methyl donor for phosphatidylcholine synthesis in the liver, and some researchers have observed a positive fat response at very high RP-Met doses, but the levels used here were apparently insufficient to shift hepatic lipid metabolism measurably [12].

From an environmental standpoint, the improved nitrogen conversion efficiency (24.1% to 28.7%) means less nitrogen excreted per litre of milk produced. In mixed crop-livestock systems where manure is recycled onto fields, this could modestly reduce nitrous oxide emissions per unit of milk protein, though a full lifecycle assessment would be needed to confirm that benefit [13, 14].

Conclusion

Kankrej cows managed on typical tropical forages and a moderate-concentrate ration are substantially limited by post-ruminal methionine and lysine supply, and correcting that

deficit with commercially available rumen-protected supplements can drive meaningful gains in milk protein output during early lactation.

The headline findings of this 90-day trial are a 33% rise in daily milk protein yield at the medium supplementation level (18 g/d RP-Met + 45 g/d RP-Lys), a statistically significant increase in milk protein percentage from 3.17% to 3.41%, and an improvement in nitrogen conversion efficiency from 24.1% to 28.7%. The response followed a curvilinear pattern, with the marginal gain between the medium and high doses falling to just 3.2 g/d of protein, suggesting that 18 g/d of RP-Met represents a practical supplementation ceiling for this breed and feeding system.

On the basis of these data, we recommend that extension services in tropical dairy regions consider incorporating RP-Met at 18 g/d and RP-Lys at 45 g/d into early-lactation feeding strategies for Kankrej and similar Zebu breeds, provided that cost-benefit analysis at current supplement prices supports adoption. Future research should extend the supplementation period beyond 90 days to assess carry-over effects on mid- and late-lactation protein yield, and should test the same dose levels in first-parity heifers and in grazing-only systems without concentrate supplementation.

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Conflict of Interest

Not available

Financial Support

Not available

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