

Digestibility, Nutritive Value and Nutrient Intake of Total Mixed Ration Incorporated with Rumen Protected and Free Lysine Methionine in Crossbred Heifer Calves

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ABSTRACT

The effect of limiting amino acids (lysine, methionine) on nutrients intake and digestibility was assessed in crossbred heifer calves in four groups of five each for 126 days. The control T₁ (control) total mixed ration (TMR) was formulated to have 14.47 % CP, 2.10 Mcal ME/kg, while T₂, T₃ and T₄ ration had 12.98, 13.24 & 12.99% CP, respectively and same 2.10 Mcal ME/kg. The T₂ TMR incorporated 6 and 2 g/kg dry matter (DM), rumen-protected lysine and methionine, respectively. In T₃ ration, the rumen protected lysine and methionine and free lysine and methionine incorporation was @ 6, 2, 3, 1 g/kg DM, and that in T₄ ration 3, 1, 3, 1 g/kg DM, respectively. The digestibility of dry matter, organic matter, crude protein, nitrogen-free extract, neutral detergent fibre, and acid detergent fibre was statistically ($p>0.05$) similar among all groups. The digestibility of ether extract and crude fibre was significantly improved in T₄ and T₃ groups of calves receiving rumen-protected and free lysine and methionine compared to control T₁, while T₂ remained intermediate. The nutritive value in terms of digestible crude protein (DCP) and actual metabolizable energy (AME) was also better of T₄ TMR. The nutrient intakes were significantly higher in lysine, methionine supplemented groups (T₂, T₃, T₄), except for digestible crude protein, which was lower, and a consistent significant value was observed in T₄ group. The supplementation of rumen-protected and free lysine-methionine (3, 1 g/kg DM TMR, respectively) in reduced protein T₄ TMR showed improved ether extract and crude fibre digestibility, superior nutritive value (DCP, AME) of total mixed ration and consistently superior intake of key digestible nutrients in crossbred heifer calves.

Key words: Crossbred calves, Digestibility of nutrients, Nutrients intake, Nutritive value, Rumen-protected and free lysine and methionine, Total mixed ration.

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INTRODUCTION

The efficient nutrition and management of dairy calves and heifers are essential, as they are the future productive stock of the dairy farm. Nutrition remains a major determinant of health, growth, and productive efficiency in dairy animals. Accelerated growth strategies have positive effects on pre-pubertal development without compromising mammary tissue growth (Akins, 2016). The efficient nutrient utilization requires balanced energy and protein supply to influence growth potential, immune function, and future milk production (Dong *et al.*, 2015). Among nutrients, proteins and their constituent amino acids (AA) play crucial roles in tissue synthesis, hormone and enzyme production, and immune responses. However, the degradability of dietary protein in rumen limits the availability of essential amino acids in dairy cattle diets (NRC, 2001).

The heifers in tropics often receive limited concentrate and low-quality roughage, resulting in poor growth, delayed puberty, and reduced lifetime productivity. The rumen microbes of growing heifers receiving lower than required

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quantity of limiting AAs, supplementation of rumen-undegradable protein or rumen-protected amino acids becomes necessary for optimum production (Merchen and Titgemeyer, 1992). Methionine serves as protein-building block, precursor of S-adenosyl methionine (SAM), the universal methyl group donor essential for DNA, RNA, and phospholipid synthesis (Lobley *et al.*, 1996; Loest *et al.*, 2002). Lysine is involved in collagen formation, carnitine production, fatty acid oxidation, and immune function (Konashi *et al.*, 2000). The lysine methionine in rumen-protected and free forms resulted in improved growth, rumen fermentation, microbial protein synthesis, and digestibility (Chung, 2003; Leonardi *et al.*, 2003; Zhang *et al.*, 2013; Metiya *et al.*, 2024). The present experiment was planned to measure effect of rumen protected and free lysine-methionine incorporation on digestibility of nutrients, nutritive value and nutrients intake of ration in crossbred heifer calves.

MATERIALS AND METHODS

This study was conducted at Livestock Research Station, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat (India). The calves were managed as per approval of Committee for Control and

Supervision of Experiments on Animals (CCSEA), New Delhi (Approval No. 429/LRS/2024, dated 15 July 2024). A total of twenty crossbred heifer calves (75% Holstein Friesian × 25% Kankrej), uniform in age, body weight, and pre-experimental growth, were randomly assigned to four dietary treatment groups, with five calves per group, for 126 days, following a 14-day adaptation. The rumen protected and free lysine and methionine were purchased from authorised commercial firms of Kemin Industries South Asia Pvt. Ltd., Ajinomoto (China) Co. Ltd., and Evonik Methionine SEA Private Ltd.

All four total mixed rations (TMRs) were iso-caloric (2.10 Mcal ME/kg), but differed in crude protein concentration and supplemental lysine and methionine (Table 1). T₁ (control) total mixed ration (TMR) contained 14.47% CP, while the TMRs T₂, T₃, and T₄ were formulated to have 12.98, 13.24 & 12.99% CP and the same energy density (2.10 Mcal ME/kg). An incorporation of rumen-protected lysine (RPL) and rumen-protected methionine (RPM) was 6 and 2 g/kg TMR DM, respectively, in T₂ TMR. The TMR T₃ was incorporated with RPL and RPM as per T₂, along with free L-lysine and DL-methionine at the rate of 3 and 1 g/kg TMR DM, respectively. An incorporation of RPL, RPM, free L-lysine and DL-methionine in T₄ TMR was 3, 1, 3, 1 g/kg DM TMR, respectively. The RPL

Table 1: Ingredient proportion (kg), composition of total mixed ration on dry matter basis and cost of TMR

Factor	Ingredients / Parameters	T1	T2	T3	T4
Ingredient proportion (kg, on dry matter base)	Compounded concentrate mixture	40.00	31.00	31.00	31.00
	Mineral vitamin premix	1.00	1.00	1.00	1.00
	Salt	1.00	1.00	1.00	1.00
	Bypass fat	0	1.75	1.75	1.75
	Rumen protected lysine	-	0.60	0.60	0.30
	Rumen protected methionine	-	0.20	0.20	0.10
	Free lysine	-	-	0.30	0.30
	Free methionine	-	-	0.10	0.10
	Wheat straw	8.00	13.70	13.30	13.70
	Groundnut straw (Gotar)	30.00	30.25	30.25	30.25
	Hybrid Napier	20.00	19.70	19.30	19.70
	Total		100.00	100.00	100.00
TMR Price (₹/kg DM)		24.84	28.96	29.81	27.28
Composition	Dry matter (%)	57.22	56.82	56.82	56.82
	Organic matter (%)	86.31	86.79	86.79	86.79
	Crude protein (%)	14.47	12.98	13.24	12.99
	Crude fibre (%)	22.37	24.86	24.86	24.86
	Ether extract (%)	3.11	4.56	4.56	4.56
	Total ash (%)	13.05	13.21	13.21	13.21
	Nitrogen free extract (%)	47.01	44.40	44.13	44.39
	Cal. ME(Mcal/kg)	2.11	2.10	2.10	2.10
	Neutral detergent fibre (%)	55.14	55.86	55.86	55.86
	Acid detergent fibre (%)	36.98	39.02	39.02	39.02



(70% lysine; LysiGem) and RPM (55% methionine; MetiPearl) were added according to treatment protocols, together with free L-lysine (78%) and DL-methionine (99%), which were diluted to match rumen-protected lysine methionine, respectively to add into TMR. The calves were individually fed *ad libitum* TMR according to their assigned dietary treatments at 10:30 and 14:00 hours daily as per weekly body weights, and increased weekly whenever leftovers were below 10%. The leftovers were collected at 07:00 hours on the following morning to determine intake. The calves were let loose for exercise in controlled conditions for two hours (8:00-10:00 h) where they had free access to fresh drinking water, and additional drinking water was provided at 12:00, 15:00, and 21:00 h at the tying place.

A seven-day digestion trial was conducted after 85 days of experimental feeding to assess dry matter and nutrient digestibility in all twenty crossbred heifer calves. Throughout the digestion trial, daily records of feed offered, leftovers, and total faecal output were recorded for each calf. TMR and leftovers were weighed each morning, and representative samples were collected, then oven-dried at $80 \pm 2^\circ\text{C}$ for 24 h, and stored in labelled bags. After completion of trial, samples were grinded through a 1.0 mm sieve for analysis. Faeces were collected quantitatively every 24 h at 08:00 h. A $1/100^{\text{th}}$ subsample of the daily faecal output was collected to determine dry matter content and to prepare acid-treated faeces for crude protein analysis. The faecal subsamples were oven-dried and finely ground to store for further analysis. For nitrogen estimation, the subsample was thoroughly mixed with commercially available sulfuric acid in a clean container, ensuring proper fixation of nitrogen for subsequent laboratory estimation and 1–2 g portions of the homogenized acid-treated faeces were used for nitrogen

estimation. Proximate composition of all TMR, leftover, and faecal samples was analysed following AOAC (1995) procedures, while fibre fractions were determined by using the method of Van Soest *et al.* (1991). The combined dataset provided the basis for calculating digestibility coefficients of dry matter and individual nutrients intake.

RESULTS AND DISCUSSION

The results on digestibility, nutritive value and digestible nutrients intake of total mixed ration are presented in Table 2 and 3.

Digestibility of Nutrients and Nutritive Value of Total Mixed Ration

The alterations in digestibility of dry matter, organic matter, crude protein, nitrogen free extract, neutral detergent fibre, and acid detergent fibre were statistically non-significant ($p > 0.05$) among all treatment groups (Table 2). The digestibility of ether extract, and crude fibre were significantly improved in T_4 group calves receiving rumen protected and free lysine and methionine. The ether extract digestibility in group T_4 and T_3 were at par with each other and showed higher digestibility than the control, while T_2 remained intermediate. These improvements indicate that the combined use of rumen-protected and free lysine–methionine may enhance rumen fermentation and overall nutrient utilisation in crossbred heifer calves. Similar to our findings, DM, OM, CP, NDF, and ADF digestibility was shown to have non-significant effect of rumen protected amino acid or N-acetyl–lysine supplementation in heifers, dairy cows, and growing calves (Broderick *et al.*, 2008; Singh *et al.*, 2015; Sai *et al.*, 2016; Duvalsaint, 2019; Tamura *et al.*, 2019; Li

Table 2: Digestibility and nutritive profile of Total Mixed Rations fed to crossbred heifer calves

Parameter		T1	T2	T3	T4	P value
Digestibility (%)	Dry matter	60.64±0.88	60.58±1.20	60.42±0.97	60.99±.46	0.989
	Organic matter	67.27±1.00	66.30±0.94	65.80±0.74	66.12±0.43	0.616
	Crude protein	65.69±0.20	62.06±1.91	64.34±0.63	63.15±0.86	0.152
	Ether extract	65.44 ^b ±0.32	66.68 ^{ab} ±0.96	68.60 ^a ±0.72	68.70 ^a ±0.55	0.009
	Nitrogen free extract	64.76±0.59	65.95±0.89	66.27±0.70	66.52±0.62	0.341
	Crude fibre	58.20 ^b ±0.74	58.89 ^b ±0.58	60.26 ^{ab} ±1.12	62.40 ^a ±0.74	0.011
	Neutral detergent fibre	52.25±1.54	51.26±1.63	54.97±1.31	54.17±0.70	0.226
	Acid detergent fibre	50.88±0.96	50.83±0.87	50.86±0.76	51.95±0.51	0.704
Nutritive value	Digestible crude protein (%)	9.51	7.72	8.00	7.86	
	Actual TDN (%)	57.55	58.83	59.80	60.30	-
	Actual ME (Mcal/kg)	2.08	2.12	2.16	2.17	

Mean with different superscripts within row, differ significantly ($p < 0.05$).

et al., 2022). However, some studies have reported improved digestibility with similar supplements (Kandil *et al.*, 2017; Gavade *et al.*, 2019).

The nutritive value of the total mixed rations (TMRs) offered to crossbred heifer calves, as assessed through the digestion trial, is presented in Table 2. The digestible crude protein (DCP) content of Control T₁ TMR was 9.51%, while a reduced and steady improvement in DCP content was observed from T₂ to T₄ and highest in T₃ (7.72, 7.86, and 8.00 %, respectively). The steady improvement in total digestible nutrients (TDN) and actual metabolizable energy (AME) from control T₁ to T₂, T₃, and highest in T₄ was observed on supplementation of rumen-protected and free lysine methionine. The greatest improvement in TDN and AME (60.30%, and 2.17 Mcal/kg, respectively) content was observed in T₄, which received a combined rumen-protected and free lysine and methionine in same dose (3 and 1 g/kg DM TMR, respectively).

Digestible Nutrient Intake

The nutrient intakes (kg/day, kg/100kg BW, and g/kg^{0.75}) of crossbred heifer calves were significantly affected by the dietary treatments (Table 3). The statistical analysis revealed significantly (p<0.01) higher DCP intake in T₁, followed by T₄ and least in T₃. DCP intake (kg/day, and g/kg^{0.75}) in T₂ was intermediate between T₃ and T₄. Those heifer calves received lysine and methionine combinations (T₂, T₃, T₄) exhibited lower values, largely due to the reduced crude protein content of these TMRs. Similar reductions in DCP intake

associated with amino acid-supplemented low-protein diets were reported in growing dairy animals (Gavade *et al.*, 2019) in Hariana heifers (Singh *et al.*, 2015), in crossbred heifers (Metiya *et al.*, 2024), and in male buffalo calves (Kandil *et al.*, 2017).

The actual metabolizable energy (AME) intake of crossbred heifer calves was highest when receiving T₄ TMR incorporated with rumen-protected and free lysine methionine. An intake of AME on percent and metabolic body weight was higher in T₄, followed by in T₁, T₂ and lowest in T₃. An intake of total digestible nutrient (TDN) by crossbred heifer calves followed the same trends. Similar improvements in TDN intake with amino acid supplementation have been reported in Jaffrabadi heifers (Movaliya *et al.*, 2013), whereas several studies documented no significant change in energy intake, including in crossbred calves (Gavade *et al.*, 2019; Metiya *et al.*, 2024), in Karan-Fries calves (Sai *et al.*, 2016), in Hariana heifers (Singh *et al.*, 2015), and in male buffalo calves (Kandil *et al.*, 2017). The supplementation of rumen-protected and free lysine methionine can enhance AME and TDN intake in a crude protein-reduced total mixed ration.

The digestible ether extract (DEE) intake as a daily, percent, and metabolic body weight was consistently higher in groups of calves received the combined rumen-protected and free lysine–methionine supplementation (T₄) and control group (T₁) than in T₃ groups having double dose of rumen protected (6, 2 g/kg DM) and free (3, 1 g/kg DM) lysine and methionine. The DEE intake on per cent and metabolic body weight was significant and in increasing order from T₁, to T₂,

Table 3: Digestible nutrient intake in crossbred heifer calves fed different TMRs

Parameter	T1	T2	T3	T4	P value
DCP (kg/day)	0.52 ^a ±0.04	0.42 ^{bc} ±0.03	0.39 ^c ±0.03	0.45 ^b ±0.03	<0.001
DCP (kg/100 kg BW)	0.33 ^a ±0.00	0.26 ^c ±0.01	0.26 ^c ±0.01	0.27 ^b ±0.00	<0.001
DCP (g/kg W ^{0.75})	11.68 ^a ±0.30	9.27 ^{bc} ±0.37	8.92 ^c ±0.22	9.77 ^b ±0.25	<0.001
AME (Mcal/day)	11.44 ^{ab} ±0.90	11.63 ^{ab} ±0.66	10.51 ^b ±0.69	12.66 ^a ±1.02	0.005
AME (Mcal/100 kg BW)	7.22 ^b ±0.14	7.22 ^b ±0.22	6.94 ^c ±0.17	7.62 ^a ±0.13	<0.001
AME(Kcal/ kg W ^{0.75})	255.29 ^b ±8.68	256.28 ^b ±8.52	242.35 ^c ±4.62	272.17 ^a ±8.56	<0.001
TDN (kg/day)	3.17 ^{ab} ±0.25	3.22 ^{ab} ±0.18	2.91 ^b ±0.19	3.51 ^a ±0.28	0.005
TDN(kg/100 kg BW)	2.00 ^b ±0.04	2.00 ^b ±0.06	1.92 ^c ±0.05	2.11 ^a ±0.04	<0.001
TDN(g/kg W ^{0.75})	70.76 ^b ±2.41	71.03 ^b ±2.36	67.17 ^c ±1.28	75.44 ^a ±2.37	<0.001
DEE (kg/day)	0.11 ^c ±0.01	0.17 ^a ±0.01	0.15 ^b ±0.01	0.18 ^a ±0.01	<0.001
DEE (kg/100kg BW)	0.07 ^d ±0.00	0.10 ^b ±0.00	0.10 ^c ±0.00	0.11 ^a ±0.00	<0.001
DEE (g/kg W ^{0.75})	3.82 ^d ±0.09	5.51 ^b ±0.13	5.11 ^c ±0.09	5.71 ^a ±0.13	<0.001
DNFE (kg/day)	1.678 ^a ±0.11	1.627 ^a ±0.07	1.450 ^b ±0.08	1.746 ^a ±0.12	0.005
DNFE (kg/100kg BW)	1.059 ^a ±0.02	1.009 ^b ±0.03	0.956 ^c ±0.02	1.049 ^a ±0.02	<0.001
DNFE (g/kg W ^{0.75})	57.75 ^a ±1.30	54.34 ^b ±1.30	50.42 ^c ±0.86	56.32 ^{ab} ±1.30	<0.001
DCF (kg/day)	0.72 ^c ±0.05	0.80 ^b ±0.03	0.73 ^{bc} ±0.04	0.90 ^a ±0.05	<0.001
DCF (kg/100kg BW)	0.45 ^d ±0.01	0.50 ^b ±0.01	0.48 ^c ±0.01	0.54 ^a ±0.01	<0.001
DCF (g/kg W ^{0.75})	27.49 ^c ±0.62	30.02 ^b ±0.72	27.85 ^c ±0.47	31.11 ^a ±0.72	<0.001

Mean with different superscripts within row, differ significantly (p<0.05).



T₃ and least in T₄. The improved DEE intake in the amino acid-supplemented groups, particularly T₄, likely reflects enhanced nutrient and energy extraction from the diet.

The digestible soluble carbohydrate/nitrogen-free extract (kg/day, kg/100kg BW, and g/kg W^{0.75}) intake of crossbred heifer calves showed a clear treatment-dependent pattern. The calves fed rumen-protected and free lysine-methionine (T₄, T₃) TMR and control T₁ ration exhibited higher DNFE intake (kg/day) than T₃ group fed double dose rumen-protected lysine methionine and single dose free lysine methionine (p<0.01). An intake on per cent body weight was significant (p<0.001) and decreased from T₄, T₁ to T₂ and least in T₃. The superior DNFE intake observed in T₄ group is likely attributable to improved dietary amino acid balance, which may have enhanced rumen fermentation and energy-yielding carbohydrate utilization, consistent with patterns observed for other energy-related intake variables in the present study. The digestible crude fibre (DCF; kg/day, kg/100kg BW, and g/kg W^{0.75}) intake was significantly affected by dietary treatments. In which, calves received the combined rumen-protected and free lysine-methionine supplement (T₄) exhibiting the highest intake across all measures (p<0.05). The control group (T₁) showed the lowest crude fibre intake, while T₂ and T₃ maintained intermediate values.

CONCLUSION

The supplementation of rumen-protected and free lysine-methionine in reduced protein TMR showed improved ether extract and crude fibre digestibility, superior nutritive value (DCP, AME) of TMR and consistently superior intake of key digestible nutrients like AME, TDN, DNFE, DEE, and DCF. Overall, among the four rations, T₄ (3, 1, 3, 1 g/kg DM TMR, rumen-protected and free lysine-methionine, respectively) consistently supported higher digestible energy, TDN, fat, and fiber intake, and higher digestibility of ether extract and crude fibre with better nutritive value of the ration. This indicated that supplementation of rumen-protected and free lysine-methionine (3, 1, 3, 1 g/kg DM TMR, respectively) was effective to improve nutrient intake, digestibility and nutritive value of total mixed ration in crossbred heifer calves.

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