RESEARCH ARTICLE

Effect of Supplementation of Rumen-Protected Methionine and Lysine on Digestibility and Rumen Fermentation in Female Calves

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Abstract

The study was performed on 12 crossbred (Kankrej × HF) female calves to evaluate the effect of supplementation of rumen-protected methionine (RPM) and lysine (RPL) on the digestibility of nutrients and rumen fermentation. The calves were randomly allotted to two equal groups T_1 and T_2 based on their body weight for 98 days . Calves in the T_1 group were fed basal TMR to meet the nutrient requirement as per ICAR (2013) standard and those in T_2 were fed basal T_1 TMR + 2 g RPM and 6 g RPL /kg DM. The digestion trial of 7 days was conducted during the 11th week of the experiment to assess the digestibility of nutrients. The rumen liquor samples were collected at 0, 2, 4, and 6 h post-feeding during the last month of the experiment to study the rumen fermentation pattern. The digestibility of nutrients was not influenced by T_2 while the fecal nitrogen excretion as a percent of nitrogen intake (N efficiency) was significantly ($p<0.05$) lower as compared to T_1. The rumen liquor soluble and total nitrogen contents were improved in T_2 TMR supplemented with RPM and RPL. However, rumen liquor pH, total volatile fatty acids, ammonia nitrogen, and non-protein nitrogen did not show any significant difference between the treatment groups. The supplementation of RPM and RPL in the diet of crossbred female calves resulted in improved rumen soluble and total nitrogen content and nitrogen use efficiency, thus preventing the detrimental effect of ammonia and greenhouse gases on the environment.

Key words: Crossbred calves, Digestibility, Rumen fermentation, Rumen-protected methionine and lysine, TMR.

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Introduction

Protein is essential for optimum growth. Two types of protein, rumen degradable protein (RDP) and undegradable protein (UDP) are required by ruminant. Ruminant's performance can be improved by dietary supplementation with rumen protected methionine (Met) and lysine (Lys). The rumen microbes rapidly degrade most amino acids into ammonia, organic acids and carbon dioxide. The produced ammonia, the main nitrogenous nutrient, is essential for bacterial growth. Therefore, the balance between RDP and UDP is very important to meet the animal's AAs requirements, otherwise extra ammonia being excreted as urea, which contributes to the atmospheric N through NO_2 emission hydrolyzed to ammonia and volatized which impact the environment. Supplementing the combination of Met and Lys effectively improved the performance of ruminants (Klemesrud *et al.,* 2000). Animals fed with high forage and maize based ration balanced with RPM either alone or incombination with RPL showed an increased digestibility of CP (Gajera *et al.,* 2013). Moreover, Noftsger *et al.* (2005) evaluated the influences of RPM on rumination in lactating cows. They noticed that the apparent digestibility levels of organic matter and *in vitro* neutral detergent fiber (NDF) of the diets fortified with RPM were higher than in control diets. However, the bacterial N entering the omasum, the concentration of ammonia and rumen volatile fatty acids profile were not altered. Hence, a study was planned to evaluate the effect of rumen-protected methionine and lysine supplementation on digestibility and rumen fermentation in crossbred female calves.

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Materials and Methods

The experiment was conducted at the Livestock Research Station (LRS) and Animal Nutrition Research Station (ANRS) of Kamdhenu University in Anand, Gujarat (India). This experiment protocol was approved by the Institutional Animal Ethics Committee Vide No. 377/LRS/2022. The experiment was carried out on 12 crossbred (Holstein Friesian × Kankrej) female calves aged 5 to 8 months. Calves were randomly divided into two equal groups of six each, based on body weight, and were allotted to treatment T_1 and T_2. The calves were dewormed before start of the experiment. Calves in the T_1 group were fed basal TMR to meet the nutrient requirement as per ICAR (2013) standard, and those in T_2 were fed basal TMR supplemented with rumen-protected methionine (RPM) @ 2 g/kg and rumen-protected lysine (RPL) @ 6 g/kg DM. The RPM and RPL were purchased from Kemin Industries. The T1 TMR consisted of a blended mixture of Concentrate mixture 45 kg, Wheat straw 15 kg, Groundnut gotar 28 kg, Green fodder (NB_{21}) 10 kg, Mineral mixture 1 kg and Salt 1 kg.

The calves were fed individually on TMR in mash form to meet their nutrient needs as per ICAR (2013) standards. The nutrient intake of all experimental calves was compared with their nutrient requirement as per ICAR (2013). The experimental calves were let loose daily for exercise in an open paddock, for two hours in the morning and one hour in the afternoon under controlled conditions during which they had free access to fresh, wholesome drinking water. The water was also offered at tying place at noon and evening.

The digestion trial of 7-days was conducted to study the digestibility of nutrients on all the experimental calves during the 11th week of experiment. The quantitative records of TMR offered, leftover and faeces were kept to assess the digestibility. The samples were dried, pooled of the 7-day collection and ground to pass through a 1 mm screen for chemical analysis. The wet acid faeces were used to estimate faecal N. The nitrogen efficiency was presented as percent faecal nitrogen to that of N intake. The rumen fermentation study was carried out during the last month. The rumen liquor was collected at 0, 2, 4 and 6 h post-feeding using negative pressure created by a suction pump. The rumen liquor was immediately strained through a four-layered muslin cloth and pH was determined. Rumen liquor was added with one mL of saturated $HgCl_2$ solution to kill the microbes and stop the metabolic activity. The nitrogen fractions like soluble, ammonical, non-protein and total as well as total volatile fatty acids were analyzed by standard Kjeldahl method. The data generated during the experiment was analyzed by a completely randomized design as per Snedecor and Cochran (1994).

Results and Discussion

Proximate Composition and Digestion Trial of TMR

The TMR (total mixed ration) offered to the experimental calves contained 15.02% crude protein, 3.01% ether extract, 21.41% crude fibre, 44.32% nitrogen-free extract, 10.02% total ash and 89.98% organic matter. The evaluation of nutritional impact of feed on animal performance highly dependents on both quantitative and qualitative attributes, assessed by the digestibility of nutrients. The digestibility coefficients, expressed as percentages, provide valuable insights into the efficiency of nutrient utilization. Table 1 presents the digestibility coefficients for key components of the diet we studied.

The digestibility of dry matter, organic matter, crude protein, ether extract, crude fibre and nitrogen-free extract was statistically at par between the T_1 and T_2 groups. However, there was numerical improvement in the digestibility of crude protein and crude fibre. These findings closely line up with studies conducted by Sai *et al.* (2016) on Karan fries calves and Gami *et al.* (2015) on Murrah buffalo calves through supplementation of rumen-protected methionine and lysine. Conversely, the outcomes of Gavade *et al.* (2019), Kandil *et al.* (2017) and Movaliya *et al.* (2013) contradicted our findings, as they observed higher digestibility of dry matter, organic matter, ether extract, crude fibre, NFE in crossbred calves, growing buffalo calves and Jaffrabadi heifers when supplemented with rumen-protected methionine and lysine.

The nitrogen excretion (g/d) was not significantly different but numerically it was lower in T_2 than T_1 fed group. The percent faecal nitrogen excretion of nitrogen intake (N efficiency) was significantly ($p<0.05$) lower in T_2 as compared to T_1 (Table 2). The supplementation of RPM and RPL prevents the detrimental effect of ammonia and greenhouse gases on the environment by improving nitrogen efficiency. Contradicting to our study Leonardi *et al.* (2003) did not find any effect on faecal N excretion by methionine

Table 1: Average digestibility coefficient (%) of nutrients during digestion trial

Nutrients	T_1	T_2	SEm	CD value
Dry matter digestibility	59.24±1.73	60.15±1.40	1.396	NS
Organic matter digestibility	66.09±1.42	66.13±0.98	1.153	NS
Crude protein digestibility	75.26±1.74	77.19±0.78	1.421	NS
Ether extract digestibility	88.25±3.78	80.55±1.70	2.564	NS
Crude fibre digestibility	47.40±3.31	51.64±4.24	3.303	NS
Nitrogen free-extract digestibility	67.27±1.36	64.54±2.63	1.814	NS

NS Non-significant.

supplementation in dairy cows. Bossche *et al.* (2023) also did not observe any effect of RPM and RPL on faecal N output.

Table 2: Average faecal nitrogen output and nitrogen efficiency in crossbred female calves

Parameters	T_1	T_2	SEm	CD value
N excretion (g/d)	35.64±2.01	33.41±0.50	1.269	NS
N intake (g/d)	143.63±7.09	149.98±2.25	4.330	NS
% Faecal N excreted as N intake	24.80^a±0.49	22.28^b±0.28	0.498	1.52

NS Non-significant, Means with superscripts a and b differ significantly (p<0.05).

Rumen Fermentation

The ruminal pH, total volatile fatty acid (TVFA), total nitrogen (N), ammonia nitrogen (NH3-N), non-protein nitrogen (NPN), and soluble nitrogen levels of experimental crossbred calves were evaluated at 0 (pre-feeding), 2,4 and 6 h post-feeding (Tables 3 to 5). The rumen pH and TVFA level indicated the rumen environment and energy availability for optimum fermentation. The various nitrogen fractions shed light on protein metabolism and microbial activity. Particularly soluble nitrogen is important for microbial utilization. The average ruminal pH and SRL TVFAs were statistically similar between groups T_1 and T_2 (Table 3). The combined effect of TVFAs and nitrogen production as well as absorption determined the rumen pH. The study on the supplementation of rumen-protected methionine and/or choline (Soltan *et al.,* 2012) and on the supplementation of rumen-protected methionine and lysine with different levels of protein diets (Benmar *et al.,* 2011) to early lactating dairy cows did not reveal any significant variation in ruminal pH. Similarly, non-significant changes in concentration of volatile fatty acids were reported by Robinson *et al.* (1999) on post-ruminal individual supplementation of methionine, lysine, and isoleucine, or all three combined in dairy cows. Whereas Soltan *et al.* (2012) reported higher TVFA concentration in early lactating cows fed a diet supplemented with rumen-protected methionine and/or choline.

Table 3: Average periodical changes in strained rumen liquor-SRL pH and TVFAs of crossbred calves

Hours of sampling	Rumen liquor pH		SRL TVFAs (mM/dL)	
	T_1	T_2	T_1	T_2
0 hr	7.05	7.04	12.17	13.63
2 hr	7.14	6.98	11.92	10.84
4 hr	6.85	7.04	12.11	11.78
6 hr	6.69	7.00	13.28	10.74
Mean±SE	6.93±0.10	7.02±0.01	12.37±0.31	11.75±0.67
	Treatment	**Period**	**Treatment**	**Period**
SEm	0.036	0.041	0.455	0.525
CD value	NS	0.117	NS	NS

NS Non-significant.

The average total-N concentration in the rumen liquor of group T_2 was significantly higher (p<0.05) compared to group T_1, but the average ammonia nitrogen concentration did not exhibit a significant difference among the two treatment groups (Table 4). The higher total nitrogen indicated improved breakdown of nitrogenous substances, and also higher microbial crude protein synthesis, as revealed by improved nitrogen utilization efficiency of crossbred female calves fed RPM and RPL in the diet. Present finding on ammonia nitrogen align with a previous study conducted by Abbasi *et al.* (2019) on Xinong Saanen goats, which aimed to assess the impact of different concentrations of rumen-protected methionine, combined with a low level of crude protein, using rumen simulation technology.

Table 4: Average total nitrogen and ammonia nitrogen concentration in strained rumen liquor

Hours of sampling	Total nitrogen (mg/dL)		Ammonia nitrogen (mg/dL)	
	T_1	T_2	T_1	T_2
0 hr	97.30	119.70	23.45	18.08
2 hr	78.40	117.37	14.70	21.70
4 hr	107.80	92.63	18.78	14.70
6 hr	97.07	125.77	19.02	22.28
Mean±SE	95.14^a±6.12	113.87^b±7.30	18.99±1.79	19.19±1.76
	Treatment	**Period**	**Treatment**	**Period**
SEm	4.737	5.469	1.137	1.313
CD value	13.433	NS	NS	NS

NS Non-significant. Means with superscripts a and b differ significantly (p<0.05).

NPN can play a role in maintaining a healthy rumen ecosystem by providing a consistent source of nitrogen for microbes to support the breakdown of fibrous materials, cellulose, and other dietary components. The availability of soluble nitrogen contributes to the overall quality protein synthesis in the rumen environment. As monitoring and managing soluble nitrogen levels optimize nutrient digestion and utilization by ruminants. In the strained rumen liquor (SRL) of crossbred calves under study, the average concentrations of non-protein nitrogen (NPN) and soluble nitrogen were higher in T_2 and than T_1 group, but statistically the difference was significant only in soluble nitrogen (Table 5). The value of soluble nitrogen coincided with the value of total nitrogen. Animals use this rapid degradable N for their growth without wastage of N as NH_3 during the fermentation process as it consists of ammonia, nitrate, amino acids, peptides, and certain true proteins (Hedqvist and Uden, 2006).

Table 5: Average non-protein nitrogen (NPN) and soluble nitrogen concentration in strained rumen liquor of calves

Hours of sampling	NPN (mg/dL)		Soluble nitrogen (mg/dL)	
	T_1	T_2	T_1	T_2
0 hr	39.20	48.53	35.93	41.07
2 hr	40.13	58.80	34.07	43.40
4 hr	45.73	43.87	30.80	42.93
6 hr	50.40	56.93	33.60	39.67
Mean±SE	43.87±2.61	52.03±3.52	33.60^{a}±1.06	41.77^{b}±0.86
	Treatment	**Period**	**Treatment**	**Period**
SEm	2.802	3.235	2.111	2.437
CD value	NS	NS	5.986	NS

NS-Non-significant. Means with superscripts a and b differ significantly ($p<0.05$)

Conclusion

The crossbred female calves fed basal TMR supplemented with rumen-protected methionine (RPM) and rumen-protected lysine (RPL) improved soluble nitrogen and total nitrogen content of rumen liquor. However, ruminal pH, TVFAs, ammonia nitrogen, and NPN did not show any significant difference among treatment groups. There was no effect of supplementation of RPM and RPL on the digestibility coefficient of dietary nutrients. The nitrogen efficiency was improved with the supplementation of RPM and RPL. Thus, supplementation of RPM and RPL prevents the detrimental effect of ammonia and greenhouse gases on the environment by improving nitrogen efficiency.

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