www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 1777-1781

www.thepharmajournal.com
Received: 23-08-2023
Accepted: 27-09-2023

Ashish Metiya
Research Scholar, Animal Nutrition Research Station, Kamdhenu University, Anand, Gujarat, India

Safi Vahora
Research Scientist, Animal Nutrition Research Station, Kamdhenu University, Anand, Gujarat, India

Ashish Patel
Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary Science & Animal Husbandry, Anand, Gujarat, India

Jigar Patel
Assistant Professor, Livestock Farm Complex, College of Veterinary Science & Animal Husbandry, Anand, Gujarat, India

Corresponding Author:
Ashish Metiya
Research Scholar, Animal Nutrition Research Station, Kamdhenu University, Anand, Gujarat, India

Effect of supplementing rumen-protected methionine and lysine with lower crude protein diet on apparent digestibility and rumen fermentation of crossbred female calves

Ashish Metiya, Safi Vahora, Ashish Patel and Jigar Patel

Abstract
The objective of this study was to explore the effect of supplementing rumen-protected methionine (RPM) and lysine (RPL) with a lower crude protein diet on apparent digestibility, nitrogen efficiency and rumen fermentation of crossbred (HF×Kankrej) female calves. In this study, twelve crossbred female calves were randomly allotted to two groups T_1 and T_2 on their body weight. The duration of the experiment was 98 days. Calves in T_1 were fed basal TMR to meet the nutrient requirement as per ICAR (2013) [7] standard and in T_2 were fed with TMR with 2% less CP than the T_1 group plus 2 g/kg DM RPM and 6 g/kg DM RPL. At the end of the experiment, the digestibility coefficient of DM was significantly ($p<0.05$) reduced in T_2 groups compared to T_1. However, the T_1 and T_2 groups did not differ with respect to the digestibility coefficient of OM, CP, EE, CF and NFE. The average DCP (%) during the digestion trial was significantly ($p<0.05$) lower in T_2 group compared to T_1. The average fecal N excretion (g/d) and N intake (g/d) were significantly ($p<0.05$) reduced in the T_2 group compared to T_1. The percent fecal nitrogen excretion as N intake (N efficiency) was significantly ($p<0.05$) lower in T_2 compared to T_1 resulting better nitrogen efficiency in reduced crude protein diet supplemented with RPM and RPL. Total volatile fatty acid and Total nitrogen concentration in strained rumen liquor were significantly decreases in T_2 compared to T_1 without affecting TDN value of the diet showing the beneficial effect of reduced CP diet supplemented with RPM and RPL.

Keywords: Crossbred calves, reduced crude protein, rumen fermentation, rumen-protected methionine and lysine, digestibility

1. Introduction
The reduction of nitrogen (N) intake is the most efficient means to improve N utilization in dairy cows, particularly helps to reduce environmental pollution (Bussink *et al.*, 1998) [4]. The supplementation of the most limiting essential amino acids (EAA) in the rumen-protected form to lower protein diets may be a successful strategy to prevent amino acid (AA) shortages for production without increasing nitrogen losses. Li *et al.* (2022) [11] and Wang *et al.* (2022) [16] found no significant effects on the digestibility of nutrients and reduced total excreta N with low crude protein diet supplementing RPM and RPL. Supplementing the RPM and RPL in steer's low CP diet (Kamiya *et al.*, 2021) [8] resulted into lower fecal N excretion. Moreover, Rostami *et al.* (2018) [13] found no any significant effect on molar proportion of volatile fatty acids and rumen pH with reduced CP diet supplementation with RPM and RPL. Keeping the above facts in mind the present study has been planned to study the effects of RPM and RPL in lowered CP diet on digestibility coefficient, nitrogen efficiency and rumen fermentation of crossbred female calves.

2. 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. This experiment protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi after a recommendation by the Institutional Animal Ethics Committee (IAEC) with reference no. 377/LRS/2022. The experiment was carried out on 12 crossbred (Holstein Friesian × Kankrej) female calves between 5 to 8 months of age. The experimental calves were kept under 1 week of adaption. Calves were randomly divided into two equal groups of six each, based on body weight. During the experiment calves were dewormed every month.

Table 1: Ingredient composition (%) of TMR offered to experimental calves

Ingredients	Basal TMR	Basal TMR with 2% less protein
Nutri power	45	39
Wheat straw	15	30
Groundnut gotar	28	18
Green fodder	10	10
Mineral mixture	01	01
Salt	01	01
Bypass fat	00	01
Total	100.0	100.0

Twelve female crossbred calves having six calves in each treatment were allotted to T_1 and T_2. Calves in the T_1 group were fed basal TMR to meet the nutrient requirement as per ICAR (2013) [7] standard and those in T_2 were fed TMR with 2% less crude protein than basal TMR plus 2 g/kg DM rumen-protected methionine (RPM) + 6 g/kg DM rumen-protected lysine (RPL). The RPM and RPL were purchased from Kemin Industries. Individual feeding of all the female calves was followed. The TMR in mash form was prepared by blending individual feed ingredients. Offered ad libitum TMR aimed to have refusals of 5 to 10%. The rumen-protected methionine and lysine were fed top-dressed to calves of the T_2 group. The nutrient intake of all experimental calves was compared with their nutrient requirement as per ICAR (2013) [7]. 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 digestion trial was conducted on all the experimental calves once during the experimental period to study the digestibility of nutrients. The collection period was of 7 days during which the representative samples of daily TMR fed, leftover and output of feces were collected and recorded. Leftovers and feces were collected every morning. Dried samples for each day of the 7 days collection period were pooled, ground to pass through a 1 mm screen and was preserved for chemical analysis. The wet feces preserved in acid was used for the estimation of fecal N.

The rumen liquor samples were collected from experimental animals at 0, 2, 4 and 6 h post-feeding through a stomach tube against negative pressure created by a suction pump. The rumen liquor was immediately brought to the laboratory and strained through a four-layered muslin cloth. Rumen pH was determined immediately after the collection of rumen liquor using a digital pH meter. After pH determination, one ml of saturated HgCl2 solution was added to each collected sample to kill the microbes and stop the metabolic activity.

The samples of strained rumen liquor (SRL) were analyzed for ammonia- N (Pearson and Smith, 1943) [12] and total-N by Kjeldahl's method. Soluble- N in the supernatant of SRL after centrifuging was estimated by Kjeldahl's method and non-protein-nitrogen was estimated by Trichloro-acetic acid precipitation of SRL and estimating the N content of supernatant by Kjeldahl's method. The concentration of total VFA was determined in SRL by the steam distillation method (Barnett and Reid, 1957) [2], using the Markham micro-distillation apparatus.

The data generated during the experiment was analyzed by a Completely Randomized Design as per Snedecor & Cochran (1994) [14].

3. Results and Discussion

3.1 Proximate Composition of TMR

Table 2: Chemical composition of TMR -I and TMR- II (On percent DM basis)

Parameter	TMR- I	TMR- II
Crude protein (%)	15.02	13.07
Ether extract (%)	03.01	03.62
Crude fibre (%)	21.41	23.21
Nitrogen-free extract (%)	44.32	44.26
Total ash (%)	10.02	09.84
Organic matter (%)	89.98	90.16

The proximate composition revealed that TMR - II has a 2% lower CP content than TMR - I as per our objectives.

3.2 Digestibility of nutrients

The evaluation of feed's nutritional value and its potential impact on animal performance is highly dependent on both quantitative and qualitative attributes, with particular emphasis on the digestibility of nutrients. The digestibility coefficients, expressed as percentages, provide valuable insights into the efficiency of nutrient utilization in both the control and treatment groups. Table 3 presents the digestibility coefficients for key components, including dry matter, organic matter, crude protein, ether extract, crude fibre and nitrogen-free extract. These coefficients serve as essential indicators to assess the nutritional worth of feed ingredients and anticipate the expected performance of animals when fed with specific types of feed. The digestibility of DM was significantly ($p<0.05$) reduced in T_2 groups compared to T_1. However, Li *et al.* (2022) [11] and Wang *et al.* (2022) [16] observed no significant difference in dry matter digestibility by reducing dietary crude protein with supplementation of rumen-protected methionine despite the difference in CP level. The T_1 and T_2 groups did not differ with respect to the digestibility coefficient of OM, CP, EE, CF and NFE. Krober *et al.* (2000) [9] also reported no significant difference in the organic matter digestibility in Brown Swiss cows by supplementing rumen-protected methionine to reduced dietary protein.

Table 3: Average digestibility coefficient (%) of nutrients during the digestion trial

Nutrients	T_1	T_2	SEM	CD Value (0.05)
Dry matter digestibility	59.24^{a}±1.73	51.39^{b}±0.94	1.396	4.21
Organic matter digestibility	66.09±1.42	64.11±1.00	1.153	NS
Crude protein digestibility	75.26±1.74	78.87±1.19	1.421	NS
Ether extract digestibility	88.25±3.78	82.58±1.60	2.564	NS
Crude fibre digestibility	47.40±3.31	49.94±2.00	3.303	NS
Nitrogen free-extract digestibility	67.27±1.36	67.66±1.05	1.814	NS

Li *et al.* (2022) [11] also observed no significant difference in crude protein, ether extract, crude protein and nitrogen-free extract digestibility by reducing dietary crude protein with supplementation of rumen-protected methionine and lysine. Wang *et al.* (2022) [16] also did not observe any significant differences in crude protein digestibility by reduction of dietary CP with supplementation of rumen-protected methionine and lysine. The average DCP (%) during the digestion trial was significantly (p<0.05) lower in T_2 (10.31±0.17) group compared to T_1 (11.30±0.29).

3.3 Fecal nitrogen output and nitrogen efficiency

The fecal nitrogen output and nitrogen efficiency is depicted in Table 4. The average fecal N excretion (g/d) and N intake (g/d) were significantly (p<0.05) reduced in the T_2 group compared to T_1. The percent fecal nitrogen excretion as N intake (N efficiency) was significantly (p<0.05) lower in T_2 compared to T_1. So, the nitrogen efficiency was better in reduce CP diet supplemented with RPM and RPL. Similar to our findings, Wang *et al.* (2022) [16] reported that total excreta nitrogen was significantly lower in all reduced protein diet groups with supplementation of rumen-protected methionine and lysine compared to the control group with a higher protein diet. In contrast, Van den Bossche *et al.* (2023) [15], Kamiya *et al.* (2021) [8] and Leonardi *et al.* (2003) [10] also did not find any significant difference in fecal nitrogen excretion with supplementation of rumen-protected methionine and lysine with low protein diet but fecal nitrogen values were numerically reduced in the low protein group diet with supplementation of rumen-protected methionine and lysine compared to the control.

Table 4: Average faecal nitrogen output and nitrogen efficiency in crossbred female calves

Parameters	T_1	T_2	SEM	CD Value (0.05)
N excretion (g/d)	35.64^{a}±2.01	29.98^{b}±0.72	1.269	3.86
N intake (g/d)	143.63^{a}±7.09	129.89^{b}±0.97	4.330	13.18
% faecal N excreted as N intake	24.80^{a}±0.49	23.10^{b}±0.65	0.498	1.52

3.4 Rumen Fermentation Pattern

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 various time points, including 0 hr (pre-feeding) and different hours post-feeding. A summary of the average values for these parameters is presented in Tables 5 to 10. These measurements provide valuable insights into the dynamic changes occurring in the rumen environment of the calves during the feeding cycle. The ruminal pH values indicate the acidity level within the rumen, while TVFA levels reflect the production of volatile fatty acids, which are essential for rumen fermentation and energy metabolism. The levels of total nitrogen, ammonia nitrogen and non-protein nitrogen shed light on the protein metabolism and microbial activity taking place in the rumen. Additionally, the measurement of soluble nitrogen offers insights into the availability of nitrogenous compounds for microbial utilization. By analyzing these parameters at different time points, a comprehensive understanding of the rumen function and nutrient utilization by crossbred calves can be obtained. Microbes in the rumen have specific pH ranges in which they thrive. Rumen fermentation is most efficient when the pH is within the optimal range for the growth and activity of these microbes. The average ruminal pH was significantly increased in the T_2 group compared to the T_1. The obtained rumen pH values were within the normal ranges reported by Hungate (1966) [6], who reported that cellulolytic bacteria need rumen pH about 6.2 to 7.0 for faster multiplication and colonization on feed particles. Fiber digestion involves a complex microbial process that generates VFAs. Adequate rumen pH is necessary for fiber-digesting microbes to thrive. Benmar *et al.* (2011) [3] in their study also did not find any significant difference in ruminal fluid pH with the supplementation of rumen-protected methionine and lysine with different levels of protein diets. Similarly, Rostami *et al.* (2018) [13] reported that reducing dietary protein with supplementation of rumen-protected methionine and lysine did not affect ruminal pH significantly (p>0.05). The decrease in pH values 2 h post-feeding in T_2 may be a result of TVFA production. Rumen pH values coincide with TVFA values of different groups. Decreasing TVFA production in the T_2 group coincides with the higher rumen pH in the T_2 group than control, this may be attributed to the proper functioning of rumen microbes.

Table 5: Average periodical changes in strained rumen liquor pH of crossbred calves

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	7.05	7.18	7.12^{a}±0.06
2 hr	7.14	6.97	7.06^{a}±0.09
4 hr	6.85	7.12	6.99^{b}±0.14
6 hr	6.69	7.06	6.88^{b}±0.19
Mean ± SE	6.93^{a}±0.10	7.08^{b}±0.04	
	T	P	T×P
SEM	0.036	0.041	0.071
CD value	0.102	0.117	0.203

The TVFA concentration in the rumen was significantly lower in the T_2 group than T_1. Volatile fatty acids are valuable products of microbial fermentation in the rumen and their values were influenced by dietary treatments along with microbial population. TVFA in rumen fermentation highlights their central role in providing energy, promoting fiber digestion, maintaining rumen health, and impacting overall feed efficiency in ruminant animals. Monitoring VFAs helps assess the effectiveness of microbial fermentation and contributes to optimizing animal nutrition and performance. In contrast to our study, Benmar *et al.* (2011) [3] in their study did not find any significant difference in total volatile fatty acids with supplementation of rumen-protected methionine and lysine with different levels of protein diets. Similarly, Rostami *et al.* (2018) [13] also reported that reducing dietary protein with supplementation of rumen-protected methionine and lysine did not affect total volatile fatty acids significantly (p>0.05).

Table 6: Average TVFA concentration (mM/dl) in strained rumen liquor

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	12.17	9.68	10.93±1.25
2 hr	11.92	10.73	11.33±0.60
4 hr	12.11	9.28	10.70±1.41
6 hr	13.28	10.84	12.06±1.22
Mean ± SE	12.37^{a}±0.31	10.13^{b}±0.39	
	T	P	T×P
SEM	0.455	0.525	0.910
CD Value	1.290	NS	NS

Total nitrogen of rumen fermentation refers to the sum of all nitrogen-containing compounds present in the feed that are subject to microbial breakdown and fermentation in the rumen. This includes both protein-bound nitrogen and non-protein nitrogen sources like ammonia and urea. The average total-N concentration in the rumen liquid of Group T_2 was significantly lower ($p<0.05$) compared to Group T_1. Total nitrogen is a reflection of rumen microbial decomposition of nitrogenous substances, utilization of ammonia and their by microbial crude protein synthesis. Monitoring and managing the total nitrogen content in the rumen is crucial for optimizing the efficiency of microbial protein synthesis and the overall digestion of nutrients. Lower total N value in T_2 coincides the lower nitrogen intake of crossbred female calves with supplemented with RPM and RPL. The reduced CP diet supplemented with RPM and RPL had reduced dry matter digestibility paralleled by lower TVFA, total N and higher pH in the rumen.

Table 7: Average total nitrogen concentration (mg/dl) in strained rumen liquor

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	97.30	87.03	92.17±5.14
2 hr	78.40	80.27	79.34±0.93
4 hr	107.80	76.30	92.05±15.75
6 hr	97.07	78.17	87.62±9.45
Mean ± SE	95.14^{a}±6.12	80.44^{b}±2.34	
	T	P	T×P
SEM	4.737	5.469	9.473
CD value	13.433	NS	NS

Ammonia nitrogen is a form of non-protein nitrogen that is produced during the breakdown of dietary proteins in the rumen through microbial fermentation. It is a key component in the rumen ecosystem as it serves as a precursor for the synthesis of microbial protein. Rumen microbes incorporate ammonia into their own protein structure, which then becomes available as a source of protein for the animal when it digests the microbes in the lower digestive tract. Controlling ammonia levels in the rumen is important to optimize microbial protein synthesis and overall ruminant nutrition. The availability of ammonia nitrogen affects the growth and activity of different microbial populations in the rumen. Proper levels of ammonia nitrogen are important to maintain a balanced microbial ecosystem. The average ammonia nitrogen concentration did not exhibit a significant difference among the three treatment groups.

Table 8: Average ammonia nitrogen concentration (mg/dl) in strained rumen liquor

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	23.45	15.05	19.25±4.20
2 hr	14.70	18.32	16.51±1.81
4 hr	18.78	17.73	18.26±0.53
6 hr	19.02	17.73	18.38±0.64
Mean ± SE	18.99±1.79	17.21±0.73	
	T	P	T×P
SEM	1.137	1.313	2.275
CD value	NS	NS	6.451

Table 9: Average NPN concentration (mg/dl) in strained rumen liquor

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	39.20	52.27	45.74±6.54
2 hr	40.13	49.47	44.80±4.67
4 hr	45.73	49.47	47.60±1.87
6 hr	50.40	41.07	45.74±4.66
Mean ± SE	43.87±2.61	48.07±2.42	
	T	P	T×P
SEM	2.802	3.235	5.604
CD value	NS	NS	NS

These findings align with a previous study conducted by Abbasi *et al.* (2019) [1] 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. In their study, authors found no significant difference in ammoniacal nitrogen concentrations.

Non-protein nitrogen (NPN) refers to nitrogen compounds in the diet that are not part of the protein structure but can still be used by rumen microbes to synthesize protein. Rumen microbes have the ability to convert NPN into microbial protein, which can then be digested by the animal. The relationship between non-protein nitrogen (NPN) and rumen fermentation is fundamental to understanding how ruminant animals utilize dietary nitrogen sources and how this process affects their overall nutrition. NPN can play a role in maintaining a healthy rumen ecosystem by providing a consistent source of nitrogen for microbes. This, in turn, supports the breakdown of fibrous materials, cellulose, and other dietary components. The average concentrations of non-protein nitrogen (NPN) in the strained rumen liquor (SRL) of crossbred calves are shown in Table 1.9. Statistical analysis revealed that there was no significant difference in the average NPN concentration among the three treatment groups. Soluble nitrogen refers to the portion of nitrogen compounds in feed that dissolves in the rumen fluid during the fermentation process. Soluble N consists of ammonia, nitrate, amino acids, peptides, and certain true proteins, supplying rapidly degradable protein and nitrogen to rumen microorganisms (Hedqvist and Uden, 2006) [5]. These compounds are available for interaction with rumen microbes and play a significant role in the overall digestive and metabolic processes within the rumen ecosystem. The availability of soluble nitrogen contributes to the overall protein content and quality of the rumen content. Proper protein availability supports animal growth and other physiological functions. Monitoring and managing soluble nitrogen levels in the rumen contribute to optimizing the digestion and utilization of nutrients by ruminant animals. The average concentration of soluble nitrogen in SRL was found non-significant between groups. Animals use this rapid degradable N for their growth without wastage of N as NH_3 as we can find coincide value of NH_3-N value in T_2. Low soluble N in a low-protein diet affected the microbiome and

metabolites, which in turn affects rumen fermentation and N utilization with reduced N excretion in the environment (Zhang *et al.*, 2022) [17].

Table 10: Average soluble nitrogen concentration (mg/dl) in strained rumen liquor

Hours of sampling	T_1	T_2	Mean ± SE
0 hr	35.93	28.47	32.20±3.73
2 hr	34.07	28.00	31.04±3.04
4 hr	30.80	29.87	30.34±0.47
6 hr	33.60	35.00	34.30±0.70
Mean ± SE	33.60±1.06	30.33±1.60	
	T	P	T×P
SEM	2.111	2.437	4.222
CD value	NS	NS	NS

4. Conclusion

Supplementation of rumen-protected methionine and lysine with a lower crude protein diet fed to crossbred female calves (T_2) resulted in significantly lower ($p<0.05$) DM digestibility, TVFA and total nitrogen concentration in rumen liquor. Feeding lower CP diets supplemented with RPM and RPL improved N efficiency and less impact on the environment by less faucal N excretion and ammonia emission.

5. References

1. Abbasi IHR, Abbasi F, Liu L, Bodinga BM, Abdel-Latif MA, Swelum AA, *et al.* Rumen-protected methionine a feed supplement to low dietary protein: effects on microbial population, gases production and fermentation characteristics. AMB Express. 2019;9(1):1-10.
2. Barnett AJG, Reid RL. Studies on the production of volatile fatty acids from the grass by rumen liquor in an artificial rumen. Journal of Agricultural Science. 1957;48:315-321.
3. Benmar HA, Rezayazdi K, Banadaki MD. Effect of supplementation of different level protein diets with rumen protected methionine and lysine on performance and ruminal parameters of Holstein dairy cows in early lactation. Iranian Journal of Animal Science. 2011;42(3):221-230.
4. Bussink DW, Oenema O. Ammonia volatilization from dairy farming systems in temperate areas: A review. Nutrient cycling in agro ecosystems. 1998;51:19-33.
5. Hedqvist H, Udén P. Measurement of soluble protein degradation in the rumen. Animal Feed Science and Technology. 2006;126(1-2):1-21.
6. Hungate RE. The rumen and its microbes. Academic Press, New York, USA; c1966. p. 533.
7. ICAR. Nutrient Requirements of Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi, India; c2013.
8. Kamiya M, Yamada T, Higuchi M. Effects of low-crude protein diets supplemented with rumen-protected lysine and methionine on fattening performance and nitrogen excretion of Holstein steers. Animal Science Journal. 2021;92(1):e13562.
9. Krober TF, Kreuzer M, Senn M, Langhans W, Sutter F. Effects of rumen-protected methionine in a low protein ration on metabolic traits and performance of early lactating cows as opposed to rations with elevated crude protein content. Journal of Animal Physiology and Animal Nutrition. 2000;84(5):148-164.
10. Leonardi C, Stevenson M, Armentano LE. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. Journal of Dairy Science. 2003;86(12):4033-4042.
11. Li Y, Wei J, Dou M, Liu S, Yan B, Li C, *et al.* Effects of rumen-protected methionine supplementation on production performance, apparent digestibility, blood parameters and ruminal fermentation of lactating Holstein dairy cows. Frontiers in Veterinary Science. 2022;9:981757.
12. Pearson RM, Smith JAB. Methods of analysis of the rumen ingest and preliminary experiments *in vivo*. Biochemical Journal. 1943;37(1):142.
13. Rostami A, Azizi O, Jahani-Azizabadi H. Effect of decreasing dietary protein and the use of rumen-protected lysine and methionine on performance of Holstein dairy cows during early lactation. Journal of Ruminant Research. 2018;6(2):43-60.
14. Snedecor GW, Cochran WG. Statistical Methods (8th Ed.). New Delhi, India: Oxford and IBH Publishing CO. Pvt. Ltd; c1994.
15. Van DBT, Goossens K, Ampe B, Haesaert G, De SJ, Boever DJL, *et al.* Effect of supplementing rumen-protected methionine, lysine, and histidine to low-protein diets on the performance and nitrogen balance of dairy cows. Journal of Dairy Science. 2023;106(3):1790-1802.
16. Wang M, Li Y, Yang Z, Shen Y, Cao Y, Li Q, *et al.* Effects of rumen-protected lysine and methionine supplementation in low-crude protein diets on lactation performance, nitrogen metabolism, rumen fermentation, and blood metabolites in Holstein cows. Animal Feed Science and Technology. 2022;292:115427.
17. Zhang Z, Shahzad K, Shen S, Dai R, Lu Y, Lu Z, *et al.* Altering dietary soluble protein levels with decreasing crude protein may be a potential strategy to improve nitrogen efficiency in Hu sheep based on rumen micro biome and metabolomics. Frontiers in Nutrition. 2022;8:815358.