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# Effect of feeding rice bran crude lecithin containing total mixed ration on rumen fermentation and blood biochemical profile in Vrindavani crossbred cattle

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## ABSTRACT

The effects of dietary supplementation with rice bran crude lecithin (RBCL) on rumen fermentation and blood biochemical parameters in crossbred cattle were investigated in the present study. For the study, 18 male crossbred Vrindavani cattle (BW 315.66±19.95 kg and 30-36 months of age) were selected and randomly divided into three groups, namely, RBCL-0, RBCL-4, and RBCL-8, containing six animals per group based on a randomized block design (RBD) and offered a total mixed ration (TMR) supplemented with 0, 4, and 8% RBCL in their concentrate mixture, respectively. The rumen *p*H was unaffected by RBCL supplementation, whereas the ammonia nitrogen ($NH_3$-N) concentration was increased in the RBCL-supplemented groups. The molar concentrations of acetate, butyrate, and TVFA were unaffected, whereas the propionate concentration increased and the A/P ratio decreased in the RBCL-supplemented groups. The activity of rumen microbial enzymes (carboxy methyl cellulase, xylanase, and avicelase) was not influenced by RBCL supplementation. The inclusion of RBCL in crossbred cattle diet did not significantly alter Hb, haematocrit, total protein, albumin, globulin, A/G ratio, BUN, ALT, AST, and total cholesterol. It can be concluded that supplementation of RBCL by replacing an equal amount of corn did not show any adverse effect on rumen fermentation and blood-biochemical profile.

**Keywords:** Blood metabolites, Crossbred, Microbial enzymes, Rice bran crude lecithin, Rumen fermentation

India is home to the world's largest livestock population, and especially the crossbred population in India increased by 26.9% over the previous census (Livestock Census 2019), due to their high-yielding capacity. High-yielding crossbred cows require energy-dense and protein-rich feeds to sustain their productivity, but our country is deficient in protein (DCP 58%) and energy (TDN 31%) rich feed ingredients (Ravi Kiran *et al*. 2012) which may cause negative energy balance (NEB) in the early lactation period. In addition, maize is the most commonly exploited source of energy in livestock diets, which creates considerable competition between humans and livestock for its use in their respective diets. Therefore, alternative energy-rich feed resources, such as agro-industrial byproducts are needed to overcome the problem of NEB in early lactation.

Rice bran crude lecithin (RBCL) is one such agro-industrial byproduct obtained during the degumming process of rice bran oil. India, being the 2nd largest producer of rice, has an enormous potential to produce and use RBCL as a source of energy for dairy cattle. It is a common practice of using lecithin as a feed additive in pre-ruminant calves (Reis *et al*. 2021), as it improves fat absorption via proper emulsification (Ullah *et al*. 2024); however, its use in ruminants has received less attention due to the prediction that it may affect rumen fermentation as a traditional fat supplement. However, few available studies have proven that feeding different types (crude, deoiled, and purified) and doses of lecithin does not adversely affect rumen fermentation (Lee *et al*. 2019); rather, it improves the fat digestion (Zhang *et al*. 2022a and b, Reddy *et al*. 2023) and rumen fermentation (Chen *et al*. 2020, Movagharnezhad *et al*. 2023) and provides additional energy to animals, which can help to overcome the problem of NEB (Tewari *et al*. 2022b, Tewari *et al*. 2023). To the best of our knowledge, studies pertaining to the effect of RBCL supplementation on rumen fermentation and the blood biochemical profile of ruminants are limited. In current study, it was hypothesized that the replacement of maize with an equal amount of RBCL in the crossbred diet may improve their energy status without compromising the rumen fermentation and metabolic profile. The objective of the present study was to evaluate the effect of feeding RBCL on rumen fermentation and blood biochemical profiles in Vrindavani crossbred cattle.

## MATERIALS AND METHODS

The present experimental procedure was approved by the

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Institutional Animal Ethics Committee (IAEC) of ICAR-IVRI, Izatnagar, as per the guidelines of the CPCSEA, New Delhi for animal experimentation with reference no. 27/06/2021-CPCSEA-DADF, dated 13 August, 2021.

*Animals, experimental design, feeding and housing management:* Eighteen adult crossbred cattle (age: 30-36 months and average BW 315.66±19.95 kg) were randomly divided following a randomized block design (RBD) into three groups consisting of six animals in each group, namely, RBCL-0, RBCL-4, and RBCL-8. The experimental animals were fed the total mixed ration (TMR) containing the roughage and concentrate mixture (70:30) in restricted feeding mode to meet their nutrient requirements as per the ICAR (2013) for a period of 120 days. RBCL was added at the rate of 0, 4, and 8% in the concentrate mixture by replacing the equal quantity of maize in the RBCL-0, RBCL-4, and RBCL-8 groups, respectively. All the experimental animals were offered daily weighed amounts of TMR and provided clean and fresh drinking water *ad lib.* in the morning and evening. The experimental animals were housed in well-ventilated sheds having individual feeding facilities. The proximate analysis of the experimental diet was performed as per AOAC (2000). The cell wall constituents such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose were estimated as per the method given by Van Soest *et al*. (1991). The calcium (Ca) and phosphorus (P) content of the feed was estimated by the method given by Talapatra *et al*. (1940).

*Rumen fermentation study:* The rumen liquor samples were collected from the experimental cattle on the 0th, 60th, and 120th day of the feeding trial by the stomach tube method, and the samples were stored at -20°C for further analysis. The *p*H of the rumen liquor was measured immediately after collection with a previously standardized electronic *pH* meter. The fraction of rumen liquor collected was stored by adding two drops of 20% $H_2SO_4$ for the estimation of ruminal ammonia nitrogen ($NH_3$-N) and was estimated as per the method described by Weatherburn (1967). Volatile fatty acids (VFA) in rumen liquor were estimated with the help of a Nucon-5765 gas chromatograph equipped with a double flame ionization detector and a glass column packed with chromosorb 101 as per the standard procedure described by Cottyn and Boucque (1968). The activity of rumen microbial enzymes such as carboxy methyl cellulase (CMCase) (nmol/min/mL), avicelase (nmol/min/mL), and xylanase (nmol/min/mL) were estimated as per the standard procedure described by Miller (1959) and Agarwal *et al.* (2002).

*Blood biochemical profile:* Blood samples (10 mL) were collected from all the experimental animals through jugular vein puncture at the beginning and end of the

Table 1. Ingredient and chemical composition (%) of experimental diet and rice bran crude lecithin (% DM basis)

| Ingredient composition (%) | Dietary treatments† | | | RBCL |
|---|---|---|---|---|
| | RBCL-0 | RBCL-4 | RBCL-8 | |
| *Roughage: Concentrate* | | | | |
| Wheat straw | 70 | 70 | 70 | |
| Concentrate | 30 | 30 | 30 | |
| *Concentrate ingredients (%)* | | | | |
| Maize | 42 | 38 | 34 | |
| Soya Bean Meal | 15 | 15 | 15 | |
| Wheat bran | 40 | 40 | 40 | |
| Mineral mixture | 2 | 2 | 2 | |
| Salt | 1 | 1 | 1 | |
| Rice bran crude lecithin | 0 | 4 | 8 | |
| Calcium carbonate ($CaCO_3$) | 0.1 | 0.15 | 0.3 | |
| *Chemical composition (% DM)* | | | | |
| Organic matter | 92.9 | 92.7 | 92.4 | 94.4 |
| Crude protein | 7.97 | 7.95 | 7.88 | 7.52 |
| Ether extract | 1.65 | 2.21 | 4.68 | 72.5 |
| Total ash | 7.14 | 7.27 | 7.63 | 5.57 |
| Total carbohydrates | 83.2 | 82.6 | 79.8 | 14.5 |
| Neutral detergent fibre | 66.0 | 65.6 | 64.6 | - |
| Acid detergent fibre | 38.9 | 37.6 | 36.2 | - |
| Hemicellulose | 27.1 | 28.0 | 28.4 | - |
| Calcium (Ca) | 1.13 | 1.21 | 1.35 | 0.54 |
| Phosphorus (P) | 0.55 | 0.67 | 0.82 | 1.21 |
| Ca: P | 2.05 | 1.81 | 1.65 | 0.45 |
| Gross energy (kcal/g) | 4.44 | 4.57 | 4.94 | 8.94 |

†, Total mixed rations supplemented without (RBCL-0) or with rice bran crude lecithin at the rate of 4% (RBCL-4) and 8% (RBCL-8) in the concentrate mixture.

trial to determine haemoglobin (Hb), haematocrit (PCV), blood glucose, total protein (TP), albumin (ALB), globulin (GLB), A/G ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (CHOL), and blood urea nitrogen (BUN) levels with the help of coral diagnostic kits (Coral Diagnostics Limited, Alto Santa Cruz, Goa, India).

*Statistical analysis:* The data obtained periodically (rumen *p*H, ammonia-nitrogen, VFA, rumen microbial enzymes, and blood biochemical parameters) was analyzed by using the general linear model of the standard statistical package of social science (SPSS software version 20.0). The means were compared using Tukey's test, and the significance was measured at $P<0.05$. P value $<0.10$ was considered a tendency.

## RESULTS AND DISCUSSION

The nutrient composition of the experimental diet, including organic matter (OM), crude protein (CP), total carbohydrates (TCHO), fibre fractions (NDF, ADF, and hemicellulose) and total ash (TA) content, were comparable among the treatment groups (Table 1). However, the ether extract (EE) and gross energy (GE) contents were slightly higher in the RBCL-supplemented group than in the control group (RBCL-0). This was because RBCL is a fat supplement and contains a higher fat (72.46% EE) end energy value (8.94 kcal/g), which was reflected in the EE and GE content of the treatment (RBCL-4 and RBCL-8) diets supplemented with RBCL. Similarly, increased EE and GE content of the ration, on supplementation of rice bran lecithin or lysophospholipids (Sontakke *et al*. 2014, Tewari *et al*. 2022b), and soy lecithin (Jenkins *et al*. 1989, Wettstein *et al*. 2000) were reported earlier.

The ruminal *p*H was comparable among the treatment groups without any significant difference ($P>0.05$). Similarly, supplementation with different types and kinds of fat and phospholipids did not alter the rumen *p*H of lactating dairy cattle (Kim *et al*. 2020, Movagharnezhad *et al*. 2023). Likewise, Jenkins *et al*. (1989) did not find any change in the rumen *p*H on supplementation of deoiled or crude soybean lecithin in sheep. Huo *et al*. (2019) also observed no change in rumen *p*H on supplementation of lysophospholipids (LPL) in the diet of lambs. In contrast, Jenkins and Fotouchi (1990) observed an increase in rumen *p*H on supplementation of soybean lecithin in the sheep diet. It was reported that the *p*H of rumen liquor was significantly higher in a phospholipid-supplemented group in male crossbred calf (Paul *et al*. 2000). Overall, in the majority of the studies, supplementation of lecithin or phospholipids or emulsifiers in ruminant diet did not cause any change in the *p*H, as the ruminal *p*H is well maintained at an optimum level between 5.5-7.0 due to well-adopted natural buffering mechanisms.

The ruminal ammonia nitrogen ($NH_3$-N) concentration was significantly higher ($P<0.05$) in the RBCL-supplemented groups than in the control group (Table 2). In disagreement with findings of present study, Jenkins and Fotouchi (1990) reported reduced $NH_3$-N concentrations in the rumen upon supplementation of soybean lecithin to sheep. Wettstein *et al*. (2000) also found similar kind of results by using different forms of canola lecithin in rumen simulation techniques. However, Movagharnezhad *et al*. (2023) did not find any change in the $NH_3$-N concentration on supplementation of LPL in early lactating HF cows, but observed a trend of increase in $NH_3$-N level, as observed in the present study. In support of present results, Jenkins (1990) observed increased rumen ammonia levels in steer-fed hydrogenated fat with added levels of lecithin in the diet. Similar results were also reported by Paul *et al*. (2000) on the supplementation of phospholipids in the diet of crossbred calves. The higher rumen $NH_3$-N in the present study may be attributed to the greater protein degradation or the proteolytic activity of rumen microbes, or alternatively less microbial protein synthesis may occur as a result of inefficient utilization of ammonia by microorganisms due to a shortage of fermentable energy sources (Cho *et al*. 2013) or the combination of both factors. The phospholipids on hydrolysis give some essential intermediates such as choline, glycerol, phosphorus, and free fatty acids, which are readily taken up by rumen microbes (Paul *et al*. 2000). The availability of these intermediate metabolites in the rumen on degradation of lecithin might have helped in the growth and proliferation of microbes, especially proteolytic bacteria, which might have increased protein degradation and resulted in an increase in the ammonia concentration in the rumen.

Table 2. Effect of dietary TMR containing different levels of RBCL on rumen metabolites and rumen microbial enzyme activity in adult crossbred cattle

| Attribute | Dietary treatments[†] | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|
| | RBCL-0 | RBCL-4 | RBCL-8 | | T | P | T*P |
| *Rumen metabolites* | | | | | | | |
| *p*H | 6.48 | 6.56 | 6.59 | 0.03 | 0.249 | 0.001 | 0.780 |
| Ammonia nitrogen (mg/dL) | 9.97[a] | 10.7[ab] | 11.6[b] | 0.23 | 0.047 | 0.189 | 0.158 |
| *Rumen microbial enzymes* | | | | | | | |
| CMCase (nmol/min/mL) | 47.6 | 44.7 | 43.2 | 1.19 | 0.327 | 0.648 | 0.691 |
| Xylanase (nmol/min/mL) | 35.2 | 34.9 | 32.4 | 0.63 | 0.146 | 0.075 | 0.820 |
| Avicelase (nmol/min/mL) | 34.6 | 30.8 | 32.2 | 0.93 | 0.258 | 0.460 | 0.812 |

[†], Total mixed rations supplemented without (RBCL-0) or with rice bran crude lecithin at the rate of 4% (RBCL-4) and 8% (RBCL-8) in the concentrate mixture. CMCase, Carboxy methyl cellulase

Table 3. Effect of dietary TMR containing different levels of RBCL on total volatile fatty acids (TVFA) and its fractions in adult crossbred cattle

| Attribute | Dietary treatments† | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|
| | RBCL-0 | RBCL-4 | RBCL-8 | | T | P | T*P |
| *Rumen volatile fatty acid concentration (mM/dL)* | | | | | | | |
| Acetate | 4.40 | 4.32 | 4.25 | 0.05 | 0.478 | 0.156 | 0.986 |
| Propionate | 1.00[a] | 1.13[b] | 1.17[c] | 0.02 | 0.002 | 0.911 | 0.363 |
| Butyrate | 0.40 | 0.39 | 0.40 | 0.01 | 0.899 | 0.003 | 0.321 |
| Iso-butyrate | 0.06 | 0.05 | 0.06 | 0.01 | 0.733 | 0.130 | 0.494 |
| Valerate | 0.01 | 0.01 | 0.01 | 0.01 | 0.501 | 0.686 | 0.820 |
| Iso-valerate | 0.04 | 0.03 | 0.03 | 0.01 | 0.221 | 0.122 | 0.200 |
| Total volatile fatty acids | 5.89 | 5.92 | 5.93 | 0.06 | 0.969 | 0.080 | 0.951 |
| A:P | 4.45[a] | 3.85[b] | 3.65[c] | 0.08 | 0.001 | 0.745 | 0.417 |

†, Total mixed rations supplemented without (RBCL-0) or with rice bran crude lecithin at the rate of 4% (RBCL-4) and 8% (RBCL-8) in the concentrate mixture.

In the present study, the activities of fibre-degrading rumen microbial enzymes such as CMCase (nmol/min/mL), xylanase (nmol/min/mL), and avicelase (nmol/min/mL) were comparable and were not influenced by RBCL supplementation in crossbred cattle (Table 2). In contrast to the present findings, Immig *et al*. (1991) reported that the free fatty acids produced upon lipolysis of dietary lipids reduced the activity of cellulose-digesting enzymes such as cellulase and CMCase by limiting enzyme-substrate attachment. However, Movagharnezhad *et al*. (2023) reported improved activity of CMCase with increased levels of LPL in the diet of lactating dairy cows. In an *in vitro* study, Kim *et al*. (2020) reported that LPL supplementation improved the enzymatic activity of bacteria in the rumen. This finding was in line with some other previous studies where the inclusion of some emulsifiers (such as Tween 80) showed positive effects on exogenous and endogenous enzymes of bacterial origin in the rumen (Lee *et al*. 2003, Wang *et al*. 2003, Hwang *et al*. 2008). In another study, it was reported that the higher levels of unsaturated fatty acids in the ruminant ration inhibit rumen fermentation by adversely affecting fibre-degrading microbes (Maia *et al*. 2010), especially cellulose-degrading bacteria (Maia *et al*. 2007). Jenkins *et al*. (1989) reported decreased fibre degradation upon supplementation of soy lecithin with higher levels of unsaturated fatty acids compared with a corn oil diet. However, in the present study, any adverse effects on fibre degradation or rumen microbial enzyme activities were not seen, which can be well correlated with the lower content of unsaturated fatty acids in rice bran crude lecithin (Adhikari and Adhikari 1986, Vandana *et al*. 2003) in comparison with other plant lecithins, such as soy lecithin, and corn lecithin (Liu *et al*. 2018, Reddy *et al*. 2008).

The concentrations of acetate, butyrate, and total volatile fatty acids (TVFA) in the rumen liquor were similar among all three groups (Table 3). However, the propionate concentration was significantly higher ($P<0.05$) in the RBCL-8 group, followed by the RBCL-4 group, than in the RBCL-0 group. The A/P ratio was reduced significantly in the RBCL-fed groups compared to the non-supplemented group (Fig. 1). Similarly, increased propionate concentrations in crossbred cattle (Paul *et al*. 2000), sheep (Jenkins *et al*. 1989) and steers (Zeleny and Kroupova 1988) fed different kinds of lecithin were reported earlier. In contrast, an increased TVFA concentration in an

Table 4. Effect of dietary TMR containing different levels of RBCL on blood biochemical profile in crossbred cattle

| Attribute | Dietary treatments† | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|
| | RBCL-0 | RBCL-4 | RBCL-8 | | T | P | T*P |
| Haemoglobin (g/dL) | 11.3 | 11.1 | 11.4 | 0.20 | 0.845 | 0.053 | 0.811 |
| Haematocrit (%) | 33.7 | 33.2 | 33.5 | 0.44 | 0.902 | 0.232 | 0.832 |
| Blood glucose (mg/dL) | 64.8 | 63.7 | 64.1 | 0.99 | 0.912 | 0.709 | 0.627 |
| Total protein (g/dL) | 6.94 | 6.65 | 6.56 | 0.07 | 0.081 | <0.001 | 0.782 |
| Albumin (g/dL) | 3.90 | 3.87 | 3.84 | 0.06 | 0.925 | <0.001 | 0.909 |
| Globulin (g/dL) | 3.04 | 2.78 | 2.72 | 0.10 | 0.425 | 0.783 | 0.909 |
| A:G ratio | 1.30 | 1.42 | 1.40 | 0.06 | 0.676 | 0.791 | 0.498 |
| BUN (mg/dL) | 18.6 | 19.5 | 21.5 | 0.78 | 0.316 | 0.002 | 0.513 |
| AST (IU/L) | 72.7 | 71.0 | 73.3 | 0.93 | 0.596 | 0.005 | 0.416 |
| ALT (IU/L) | 23.2 | 23.3 | 24.3 | 0.72 | 0.782 | <0.001 | 0.917 |
| Cholesterol (mg/dL) | 108.7 | 116.4 | 117.4 | 2.93 | 0.428 | <0.001 | 0.076 |

†, Total mixed rations supplemented without (RBCL-0) or with rice bran crude lecithin at the rate of 4% (RBCL-4) and 8% (RBCL-8) in the concentrate mixture. BUN, Blood urea nitrogen; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

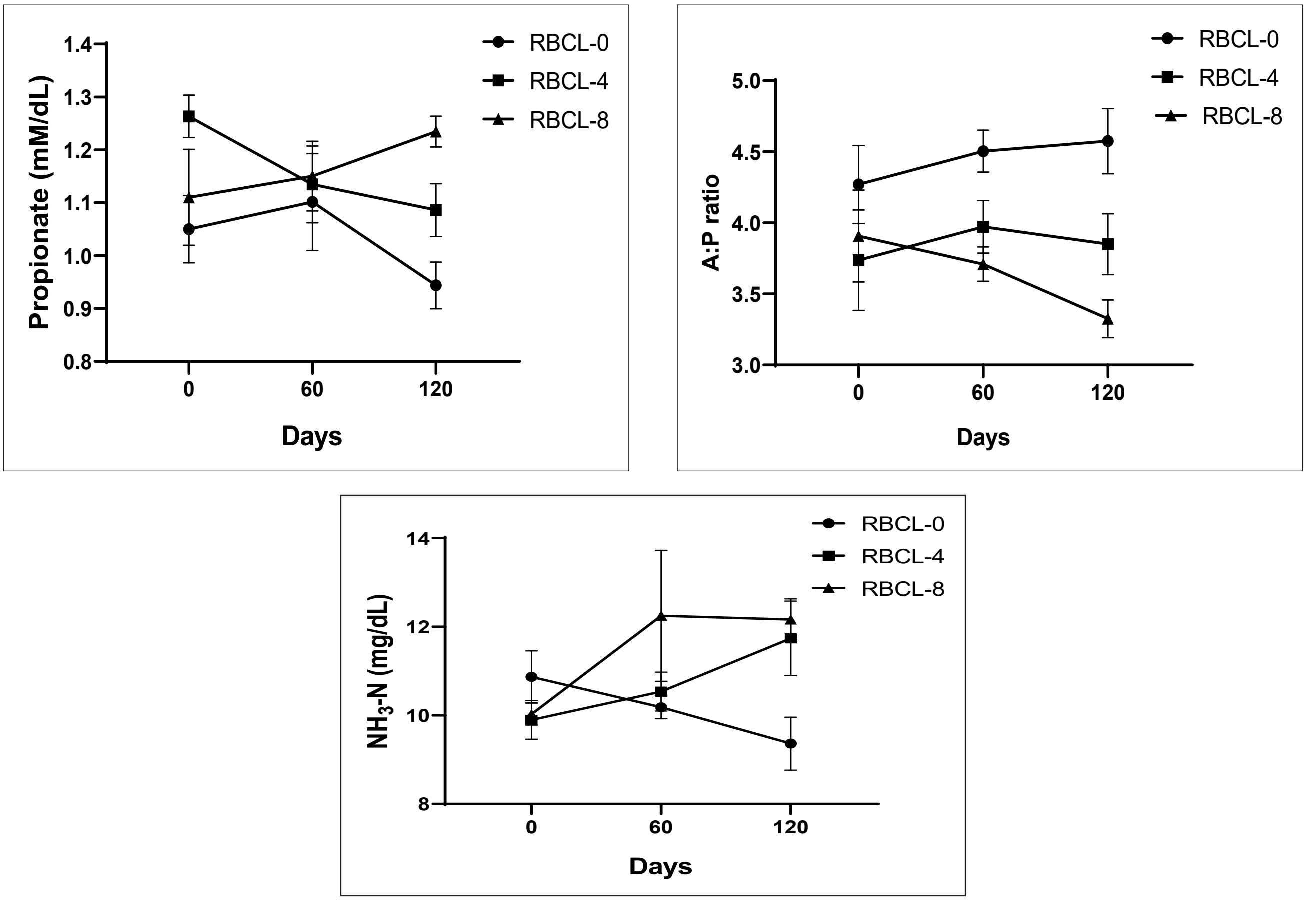


Fig. 1. Effect of RBCL supplementation on rumen fermentation metabolites in crossbred cattle.

*in vitro* study conducted with supplementation of deoiled soybean lecithin was reported by Jenkins *et al*. (1989). The increased level of LPL in the diet caused an increase in the molar concentration of butyrate and TVFA without showing any effect on acetate, propionate and the A/P ratio in beef cattle (Zhang *et al*. 2022b). In another study, Jenkins and Fotouchi (1990) found a reduction in TVFA on feeding 5.2% lecithin in the diet of sheep. Paul *et al*. (2000) observed an increase in TVFA concentration upon feeding phospholipids to crossbred calves. In disagreement with findings of present study, Zinn (1989) observed increased acetate: propionate (A/P) ratio upon supplementation of lecithin to feedlot steers. In another study, Movagharnezhad *et al*. (2023) reported that increased levels of LPL in the diet of lactating cows resulted in increased concentrations of ruminal acetate and valerate without affecting propionate or the A/P ratio. In support of findings of present study, a reduction in the A/P ratio was also reported by Tewari *et al*. (2023) upon supplementation of RBCL to crossbred lactating cows and Cho *et al*. (2013) also observed similar kind of results in an *in vitro* rumen fermentation study. In the present study, the increase in propionate concentration in the RBCL-fed group might be due to the fermentation of glycerol that is produced in the rumen on hydrolysis of lecithin (Garton *et al*. 1961), and it might also result from the fermentation of the total carbohydrate content of crude RBCL. The increased molar concentration of propionate might be responsible for the reduced A/P ratio in the RBCL-supplemented groups.

The haematological parameters such as haemoglobin (Hb) and haematocrit (PCV) were unaffected by RBCL supplementation in the experimental animals. Serum biochemical parameters such as albumin, globulin, A:G ratio, BUN, AST, ALT, and cholesterol were not influenced by RBCL supplementation in the experimental animals (Table 4). However, a decreased tendency (P=0.081) of serum total protein (TP) in the RBCL-4 and RBCL-8 groups was observed in the present experiment. Similar to these findings, Jenkins *et al*. (1989) reported that the supplementation of different forms of soy lecithin did not have any effect on serum glucose, cholesterol, and triglycerides in adult sheep. In another study, Reis *et al*. (2021) evaluated the supplementary effect of lysolecithin on blood metabolites such as glucose, albumin, creatinine, triglycerides, and cholesterol in HF dairy calves and reported that supplementation did not alter any of the tested metabolites except total protein, which improved on lecithin supplementation. In contrast, increased concentrations of serum triglycerides, and cholesterol were observed upon supplementation with soy lecithin and rice bran crude lecithin in Hanwoo steers and crossbred calves, respectively (Li *et al*. 2017, Tewari *et al*. 2022b). Contrastingly, LPL supplementation in beef cattle has shown to decrease total cholesterol and triglycerides and increase total protein and albumin levels (Zhang *et al*. 2022a). The discrepancies with respect to the blood biochemical profile in various studies could be due to the different forms, doses of lecithin, and animal models that were used for experiments.

The composition of the basal diet may also influence the effect of the fat source on ruminal fermentation. Fats that normally inhibit fermentation and digestion often cause less inhibition when the fibre content of the basal diet is high (Jenkins 1993). The greater number of fibre particles in the rumen might provide binding sites for fatty acids and reduce their attachment and toxic effects on microbial cells. Therefore, the growth and normal function of rumen microbes were unaffected. In the present study, a 70:30 roughage and concentrate ratio was used, which might also be a reason for the lack of adverse effects on rumen fermentation on the supplementation of RBCL in cattle. In addition, the effects of supplementary phospholipids on rumen fermentation differ depending on the level and kind of phospholipids used in the study.

In the present study, supplementation with rice bran crude lecithin increased rumen ammonia nitrogen, improved propionate concentration, and reduced the acetate:propionate ratio without adversely affecting the blood biochemical profile of crossbred cattle. Hence, it may be concluded that rice bran crude lecithin can be added up to 8% in the concentrate mixture by replacing an equal amount of corn in the diet of crossbred cattle without showing any adverse effects on rumen fermentation and the blood biochemical profile.

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