



Evaluation of Dietary Rice Bran Crude Lecithin as Substitute of Maize Grain in the Ration of Crossbred Calves

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ABSTRACT

The aim of the present experiment was to assess the potential of rice bran crude lecithin (RBCL) as an alternative energy source in the diet of growing cattle. Two experiments were conducted: in the first experiment total gas and methane production along with digestibility of dry and organic matter was measured *in vitro*; in the second experiment, two different experimental levels of RBCL was tested on eighteen crossbred calves. Six substrate comprising of either 0 (T₁), 4 (T₂), 8 (T₃), 12 (T₄), 16 (T₅) or 20 (T₆) % RBCL, replacing equal amount of maize grain of control were tested. Response criteria during *in vitro* evaluation was gas and methane production, and digestibility of dry matter and organic matter. During the second experiment, eighteen crossbred calves were randomly distributed into three groups of six each in an experiment based on completely randomized design. Calves of control group (GI) were fed a standard diet of concentrate and wheat straw to meet their nutrient requirements (ICAR, 2013). However, 8 and 12% of maize of the concentrate mixture of control group was replaced with RBCL in groups GII and GIII, respectively. The net gas production and methane production (ml/200 mg), *in vitro* true dry matter and organic matter digestibility (%) decreased (P<0.001), while, efficiency of microbial biomass production (%) increased (P<0.001) with the increased level of RBCL. During *in vivo* study digestible dry matter and organic matter intake (g/kgW^{0.75}) was significantly (P<0.05) lower in GIII as compared to GI. The intake of total digestible nutrients and digestible crude protein was comparable (P>0.05) among the groups. Although body weight gain apparently seemed to decrease with increased levels of RBCL values were comparable among the groups. Clinical chemistry profile was comparable among the groups. It was concluded that RBCL can replace up to 12% of maize grain of the concentrate mixture without any adverse impact on plane of nutrition, clinical chemistry and performance of crossbred calves.

Key words: Clinical chemistry, Cortisol, *In vitro*, Plane of nutrition, Rice bran crude lecithin

INTRODUCTION

Considering the deficit of concentrates, animal nutritionists are continuously looking for alternate feed sources that can supply critical nutrients. During refining process of rice bran oil, gummy materials are removed by degumming process, this mixture of gummy byproducts is commonly known as rice bran crude lecithin (RBCL). It amounts to roughly 1.5-2% of oil (Jala and Prasad, 2015), making RBCL easily available in good amounts. The active portion of rice bran lecithin is a complex mixture of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) as major phospholipids, along with glycolipids, triglycerides, free fatty acid, oryzanol, tocopherols, sterols and waxes as minor components (Adhikari and Adhikari, 1986). Feeding of raw, standardized, liquid, non-fat and/

or modified lecithin did not have any adverse effect on rumen fermentation (Jenkins *et al.*, 1989). Sontakke *et al.* (2014b) revealed that rice bran lyso-phospholipids supplementation did not show any adverse impact on the performance of the dairy cows. Recently, several research workers have studied the effect of dietary lysophospholipid (LPL) on performance of ruminants including sheep (Huo *et al.*, 2019), beef cattle (Song *et al.*, 2015) and dairy cows (Rico *et al.*, 2017; Lee *et al.*, 2019), but the responses of LPL are inconsistent. Therefore, the present study was conducted to investigate the effect of replacement of maize grain of concentrate mixture with graded levels of RBCL on *in vitro* fermentation, *in vivo* assessment of plane of nutrition, growth performance and clinical chemistry profile in crossbred calves.

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MATERIALS AND METHODS

Animal care procedures were approved (approval number: 25/2/2020-CPCSEA-DADF, dated 13 April, 2020) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

For *in vitro* study, six substrates (roughage: concentrate, 50:50) comprising either 0 (T₁), 4 (T₂), 8 (T₃), 12 (T₄), 16 (T₅) or 20 (T₆) % RBCL (Table 1) were formulated. *In vitro* gas production was measured following the method of Menke and Steingass (1988).

The 200 mg substrate (100 mg wheat straw: 100 mg concentrates containing graded level of RBCL replacing maize) was weighed in plastic boat and transferred into the bottom of glass syringe. The *in vitro* medium was prepared by mixing 500 ml of distilled water, .125 ml of micro minerals solution, 250 ml rumen buffer solution and 250 ml macro minerals solution, 1.25 ml resazurin solution, and 50 ml reducing solution

(prepared fresh and added just prior to adding rumen liquor). The medium was pre-warmed to 39°C and bubbled with CO₂ just before inoculation of rumen liquor. Rumen liquor was collected from fistulated adult male cattle maintained on a roughage based diet before morning feeding and watering into a pre-warmed thermos flask and immediately brought to the laboratory. Just after mixing the prepared medium and rumen liquor, 30 ml of the incubation medium was injected to the syringes. The syringes were shaken gently and residual air or air bubbles were removed and outlet was closed. Each substrate was incubated in triplicate in three different run that generated nine measurements per substrate sample. After 24 h of incubation, the volume of gas production was recorded and a suitable aliquot of gas was withdrawn from the tip of the incubation syringes using gas tight syringe and analysed for methane using Gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with *porapak-Q*. *In vitro* true digestibility (IVDMD) and *in vitro* true organic matter digestibility (IVOMD)

Table 1. Chemical composition of various concentrate mixtures and wheat straw used as substrates

| Attributes (%) | Concentrate mixtures | | | | | | Wheat straw |
|--|----------------------|----------------|----------------|----------------|----------------|----------------|-------------|
| | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ | T ₆ | |
| Ingredients (%) | | | | | | | |
| Maize | 42 | 38 | 34 | 30 | 26 | 22 | |
| Soybean meal | 25 | 25 | 25 | 25 | 25 | 25 | |
| Wheat bran | 30 | 30 | 30 | 30 | 30 | 30 | |
| Mineral mixture | 2 | 2 | 2 | 2 | 2 | 2 | |
| Salt | 1 | 1 | 1 | 1 | 1 | 1 | |
| Rice bran crude lecithin (RBCL) | 0 | 4 | 8 | 12 | 16 | 20 | |
| Chemical composition (% dry matter) | | | | | | | |
| Dry matter | 87.34 | 87.80 | 88.24 | 88.86 | 89.38 | 89.95 | 90.07 |
| Organic matter | 93.64 | 93.65 | 93.57 | 93.10 | 93.35 | 93.11 | 92.03 |
| Crude protein | 20.39 | 20.24 | 20.13 | 19.94 | 19.80 | 19.48 | 3.71 |
| Ether extract | 2.97 | 5.56 | 7.84 | 10.51 | 13.36 | 16.25 | 1.33 |
| Total ash | 6.36 | 6.35 | 6.43 | 6.90 | 6.65 | 6.89 | 7.97 |
| Neutral detergent fibre | 15.91 | 15.66 | 15.26 | 14.92 | 14.00 | 14.50 | 71.48 |
| Nitrogen free extract | 53.76 | 52.19 | 50.34 | 47.73 | 46.19 | 42.88 | 15.51 |

were estimated following the procedure of Blümmel and Lebzein (2001).

For *in vivo* study, after acclimatization, eighteen crossbred male calves (*Bos taurus* x *Bos indicus*), of same age, were randomly divided into three groups (GI, GII and GIII) of six animals each, following Completely Randomized Design (CRD). Calves of the control group (GI) were fed a standard diets of concentrates and wheat straw to meet their nutrient requirements (ICAR, 2013). However, 8 and 12% of maize of the concentrate mixture of control group was replaced with RBCL in groups GII and GIII, respectively. A growth cum feeding trial was conducted for 120 days under similar managemental conditions in the animal shed of Animal Nutrition Division, ICAR-Indian Veterinary Research Institute, Izatnagar. Prior to initiation of the experimental trial, animals were treated for ecto- and endo-parasites as per the standard protocol. About 1.50 kg of green fodder was given to all the experimental calves to satisfy the part of their respective requirements of vitamins. Fresh and clean drinking water was made available *ad libitum* twice a day.

The calves were individually offered measured quantity of concentrate and wheat straw in the morning (9:30AM). Feed offered and refusal of ration from all calves were weighed daily and sampled at weekly intervals for subsequent analysis of dry matter (DM) to assess the average daily dry matter intake (DMI) during the experimental period. For growth study, the weight of individual calve was recorded at fortnightly intervals in the morning before feeding and watering for four months in order to assess the changes in body weight and average daily gain (ADG). The plane of nutrition was calculated using data of a metabolism trial of 8 days duration including 2 days of adaptation in metabolic cages followed by 6 days collection. Blood samples were collected on start (0 d) and end (120 d) of experiment by puncture of jugular vein. Out of 10 ml of collected blood, 4 ml was dispensed into gel containing serum vacutainers and allowed to clot at room temperature for 45 min. The collected sera samples were stored in deep freeze (-20°C) for further serum-biochemical analysis. The concentration of

haemoglobin in freshly collected blood was estimated as per Oser (1979) and haematocrit value was estimated as per Jain (1986). Serum concentration of alanine amino transferase (ALT) and aspartate transferase (AST) were estimated by using commercial diagnostic kits (Coral clinical system, India). Serum concentration of cortisol and testosterone were estimated by using commercial ELISA kits (LDN IMMUNOASSAY AND SERVICES, Germany) as per manufacturer's recommendations. Cortisol and testosterone ELISA kit were based on the principle of competitive binding. The microtiter wells were coated with a monoclonal antibody directed towards an antigenic site on the cortisol or testosterone molecule. Endogenous cortisol or testosterone of a sample compete with a cortisol or testosterone -horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of cortisol or testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of cortisol or testosterone in the sample.

Feed and faecal samples were analyzed for proximate principles like DM, organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen free extract (NFE) using standard method (AOAC, 2012). Feed and faecal samples were also analyzed for neutral detergent fibre (NDF) content (Van Soest *et al*, 1991). Data were analysed using General linear model of ANOVA by using SPSS computer package (SPSS version 20.0, SPSS Inc.). The means were compared using Duncan's multiple range test (Snedecor and Cochran, 1994). Significant difference among treatments was established at $P < 0.05$.

RESULTS AND DISCUSSION

The results of *in vitro* gas production (IVGP) of substrate containing different levels of RBCL are presented in Table 2. Total gas production (TGP) and methane production (ml/200 mg) decreased significantly ($P < 0.001$) with increasing level of RBCL. *In vitro* digestibility of DM (IVDM) and organic matter (IVOM)

Table 2. *In vitro* fermentation pattern on various substrates containing graded level of RBCL

| Attributes | Treatments* | | | | | | SEM | P value |
|--|--------------------|---------------------|----------------------|---------------------|--------------------|---------------------|------|---------|
| | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ | T ₆ | | |
| TGP (ml/200 mg) | 19.13 ^a | 18.51 ^{ab} | 18.11 ^{abc} | 17.42 ^{bc} | 16.89 ^d | 16.98 ^{cd} | 0.19 | 0.002 |
| CH ₄ production (ml/200 mg) | 4.04 ^a | 3.90 ^a | 3.81 ^{ab} | 3.60 ^{bc} | 3.46 ^c | 3.46 ^c | 0.05 | 0.000 |
| IVDMD (%) | 69.10 ^a | 68.97 ^a | 68.62 ^a | 67.51 ^a | 64.74 ^b | 64.83 ^b | 0.43 | 0.000 |
| IVOMD (%) | 67.10 ^a | 67.02 ^a | 66.83 ^a | 65.78 ^a | 62.94 ^b | 63.11 ^b | 0.44 | 0.002 |
| Partition factor (mg/ml) | 6.31 | 6.58 | 6.68 | 6.91 | 6.82 | 6.67 | 0.09 | 0.568 |
| MBP (mg) | 72.44 | 73.42 | 74.14 | 74.56 | 74.03 | 74.78 | 0.49 | 0.841 |
| EMP (%) | 53.98 ^c | 54.79 ^{bc} | 55.47 ^{bc} | 56.68 ^b | 58.83 ^a | 59.22 ^a | 0.39 | 0.000 |

TGP, total gas production; IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; MBP, microbial biomass production; EMP, efficiency of microbial production; ^{a,b,c,d}Mean values within a row are significantly different (P<0.05)

were significantly (P<0.001) higher in groups T₁, T₂, T₃ and T₄ as compared to T₅ and T₆ groups. Partition factor (PF, mg/ml) and microbial biomass production (MBP, mg) were comparable among all treatment groups. The efficiency of microbial protein synthesis (EMP, %) was significantly (P<0.001) higher in T₆ group followed by T₅, T₄, T₃, T₂ and T₁, respectively.

Gas production in rumen depends upon the microbial degradation of feedstuffs by solubilization and

fermentation process. Both processes depend upon the chemical composition and structure of substrate. The TGP, methane production, IVDMD and IVOMD were not increased after RBCL inclusion in current study. It may be attributed to the lower soluble carbohydrate available to the microbes for fermentation as maize was replaced by rice bran crude lecithin. Contrary to above findings, Sontakke *et al.* (2014a) found that total gas production, IVDMD and IVOMD increased upto 6%

Table 3. Effect of graded levels of rice bran crude lecithin on feed intake (kg dry matter), feed conversion ratio and feed cost during 120 days of trial

| Attributes | Dietary treatments | | | SEM | P value |
|-----------------------------------|----------------------|-----------------------|----------------------|--------|---------|
| | GI | GII | GIII | | |
| Voluntary Feed intake (kg) | | | | | |
| Concentrate | 302.18 | 307.46 | 308.25 | 3.90 | 0.556 |
| Wheat straw | 197.59 ^a | 181.68 ^{ab} | 131.56 ^b | 12.06 | 0.055 |
| Green fodder | 36.00 | 36.00 | 36.00 | 0.00 | 0.000 |
| Total | 535.76 | 525.14 | 475.81 | 15.11 | 0.237 |
| Feed conversion ratio | 5.43 | 5.73 | 5.44 | 0.20 | 0.916 |
| Roughage: Concentrate | 43:57 | 41:59 | 35:65 | - | - |
| Feed cost (₹) | | | | | |
| Concentrate cost** | 9655.71 | 9713.02 | 9737.90 | 141.16 | 0.937 |
| Roughage cost | 2517.24 ^a | 2411.21 ^{ab} | 2077.09 ^b | 80.42 | 0.055 |
| Total feed cost | 11872.95 | 11824.23 | 11514.99 | 201.08 | 0.746 |
| Feed cost/kg gain | 120.51 | 129.20 | 132.41 | 4.41 | 0.530 |

Calves of the control group (GI) were fed a standard diets of concentrates and wheat straw to meet their nutrient requirements (ICAR, 2013). However, 8 and 12% of maize of the concentrate mixture of control group was replaced with RBCL in groups GII and GIII, respectively.

level and then decreased while the PF and MBP did not show any change, and methane production decreased with increasing the level of rice bran lysophospholipid inclusion. The total gas production improved with increasing the level of lysophospholipids (Cho *et al.*, 2013). Paul (1994) recorded increase in gas production at 4% level of pure phospholipids in an *in vitro* study. The results clearly indicate that upto 12% level of inclusion there was no significant decrease in *in vitro* dry and organic matter digestibility. Thus, RBCL can be incorporated upto 12 % in the ration. Added advantage is that at that level there was also reduction in methane production. Hence, for *in vivo* study, three levels of RBCL *i.e.* 0, 8 and 12% was selected.

The data pertaining to voluntary feed intake, FCR and feed cost are shown in Table 3. Dry matter intake through concentrate was comparable among the groups which indicate that RBCL is palatable. However, intake of wheat straw decreased significantly ($P < 0.10$) with increasing level of RBCL. Total DMI (kg) and DMI/d

(g/d) was lower by 2% in group GII fed RBCL at 8% and by 11% in GII fed RBCL at 12% compared to control (GI) group. The FCR was comparable while roughage concentrate ratio tended to be wider with increased inclusion of RBCL. Total feed cost tended to decrease, however feed cost/kg gain tended to increase with increase in the inclusion level of RBCL. Data on fortnightly BW gain and DM intake is presented in Fig. 1. The BW gain was linearly increased with the period, however comparable between all dietary groups. The BW gain was lowest in GIII, highest in GI, while intermediate in GII group. The final body weight of GI group (control) was 3% higher than GII and 6% higher than GIII group calves. The DM intake in group fed 12% RBCL was significantly lower than other two groups.

The results of the present study are compatible with the findings of various previous workers (Sontakke *et al.*, 2014b; Rico *et al.*, 2017; Huo *et al.*, 2019; Lee *et al.*, 2019), who observed no significant difference in

Table 4. Effect of various dietary levels of rice bran crude lecithin on nutritive value and plane of nutrition

| Attributes | Dietary treatments | | | SEM | P value |
|----------------------------|--------------------|---------------------|--------------------|-------|---------|
| | GI | GII | GIII | | |
| Nutrient intake | | | | | |
| Digestible dry matter | | | | | |
| g/d | 2767.32 | 2633.93 | 2363.34 | 99.36 | 0.187 |
| g/kgW ^{0.75} | 55.35 ^a | 53.56 ^{ab} | 49.78 ^b | 1.00 | 0.068 |
| Digestible organic matter | | | | | |
| g/d | 2688.38 | 2533.55 | 2278.31 | 95.33 | 0.154 |
| g/kgW ^{0.75} | 53.77 ^a | 51.52 ^a | 48.00 ^b | 0.95 | 0.033 |
| Digestible crude protein | | | | | |
| g/d | 443.09 | 447.63 | 433.67 | 8.56 | 0.806 |
| g/kgW ^{0.75} | 8.92 | 9.17 | 9.18 | 0.20 | 0.857 |
| Total digestible nutrients | | | | | |
| g/d | 2776.30 | 2762.21 | 2570.84 | 81.29 | 0.601 |
| g/kgW ^{0.75} | 55.54 | 56.21 | 54.40 | 0.88 | 0.766 |
| Nutrient density (%) | | | | | |
| Digestible crude protein | 10.23 | 10.63 | 11.40 | 0.27 | 0.217 |
| Total digestible nutrients | 63.49 | 65.08 | 67.95 | 1.03 | 0.198 |

Calves of the control group (C) were fed a standard diets of concentrates and wheat straw to meet their nutrient requirements (ICAR, 2013). However, 8 and 12% of maize of the concentrate mixture of control group was replaced with RBCL in groups GII and GIII, respectively.

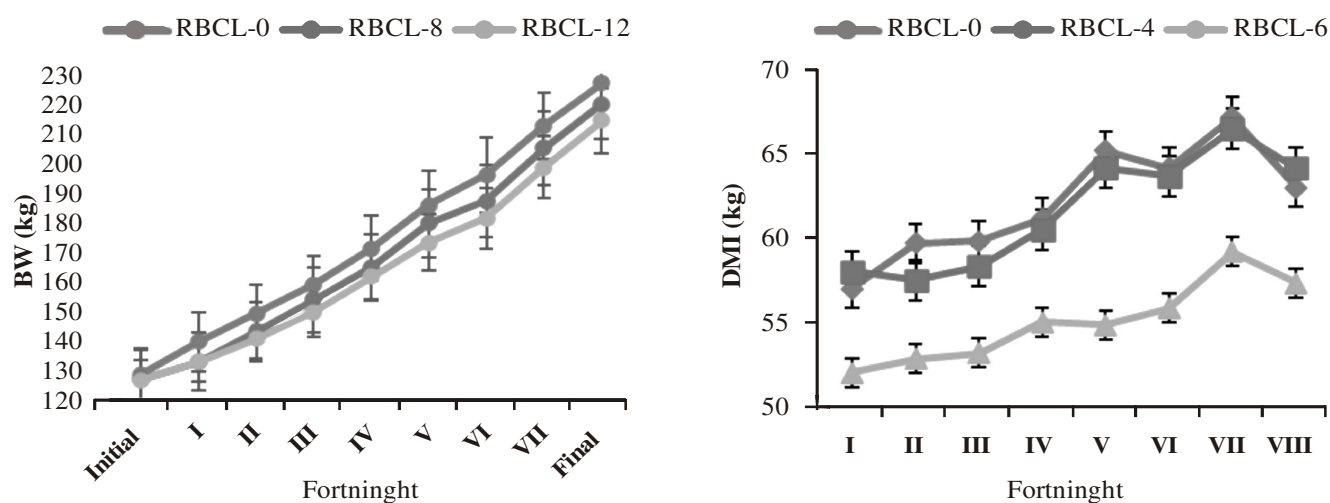


Fig. 1. Effect of graded levels of RBCL on fortnightly body weight gain dry matter intake in calves

dry matter and nutrient intake on supplementation of lecithin in lactating cows. The lower fortnightly body weight of calves fed 8 and 12% RBCL could be related to the lower DM and nutrient intake along with decrease rumen fermentation. Digestible DM and DOM intake ($\text{g/kgW}^{0.75}$) were significantly ($P < 0.05$) lower in group III as compared to GI, while group II was in intermediate position. The TDN and DCP intake ($\text{g/kgW}^{0.75}$) were comparable among dietary groups. The nutrient density in terms of DCP and TDN were 4 and 2 % higher in GII & 11 and 7 % higher in GIII group as compared to control (Table. 4). The DCP and TDN intake was comparable among dietary groups. In the conformity, Sontakke *et al.* (2014b) reported no difference in CP and TDN intake due to RBLPL

supplementation.

The effect of dietary levels of RBCL on hematology (Hb, haematocrit), liver profile (ALT, AST), stress (cortisol) and reproductive performance (testosterone) were assessed and presented in Table 5. The Hb (g/dl) and haematocrit (%) values were comparable irrespective of dietary treatments, however values were significantly ($P < 0.05$) higher on 120 d as compared to 0 d. The mean values of serum enzymes *viz.*, AST and ALT in calves did not differ significantly ($P > 0.05$), while these values were significantly ($P < 0.05$) higher at 120 d as compared to 0 d. The treatment mean of cortisol (ng/ml) was tended to decrease with the increasing level of RBCL. The period mean of cortisol (ng/ml) was lower numerically at 120 d in comparison

Table 5. Effect of various dietary levels of rice bran crude lecithin on blood profile

| Index | Treatment | | | SEM | Sampling time | | SEM | P value | | |
|----------------------|-----------|-------|-------|-------|--------------------|--------------------|-------|---------|-------|-------|
| | GI | GII | GIII | | 0 d | 120 d | | P | T | P*T |
| Hb (mg/dl) | 8.53 | 8.48 | 8.48 | 0.12 | 7.89 ^b | 9.12 ^a | 0.09 | 0.001 | 0.928 | 0.802 |
| Haematocrit (%) | 30.13 | 30.00 | 29.50 | 0.72 | 27.75 ^b | 32.00 ^a | 0.59 | 0.001 | 0.812 | 0.812 |
| ALT (IU/L) | 17.70 | 22.05 | 32.10 | 2.41 | 18.05 ^b | 23.85 ^a | 1.97 | 0.052 | 0.270 | 0.721 |
| AST (IU/L) | 70.71 | 69.99 | 71.17 | 4.71 | 65.01 ^b | 76.10 ^a | 3.84 | 0.056 | 0.928 | 0.986 |
| Cortisol (ng/ml) | 91.10 | 90.00 | 76.00 | 18.76 | 94.81 | 76.59 | 15.32 | 0.411 | 0.819 | 0.749 |
| Testosterone (ng/ml) | 2.84 | 2.67 | 2.81 | 0.16 | 2.82 | 2.73 | 0.13 | 0.620 | 0.701 | 0.480 |

Calves of the control group (C) were fed a standard diets of concentrates and wheat straw to meet their nutrient requirements (ICAR, 2013). However, 8 and 12% of maize of the concentrate mixture of control group was replaced with RBCL in groups GII and GIII, respectively; ^{ab}Means with different superscripts within a row differ significantly; ^{xy}Means with different superscripts within a column differ significantly

to 0 d. The serum testosterone (ng/ml) concentration among groups was non-significant ($P>0.05$) irrespective of period, treatment and period treatment interaction.

The haematological profile (Hb and haematocrit) is considered to be an indicator of normal health status of the animals. The Hb (g/dl) and haematocrit (%) values were within the normal physiological range (Kaneko *et al.*, 2008). RBCL supplementation showed no adverse impact on haematological profile. ALT is the enzyme normally found in hepatocytes only, whereas, AST is present in hepatocytes, RBC and in cardiac and skeletal muscle. These enzymes are considered as biomarkers of hepatic affections (Evans, 2009) and their activity is an indicator of damage to liver and muscle (Casteel, 1999). The mean values were within the suggested normal physiological range (Kaneko *et al.*, 2008). The results are in accordance with Huo *et al.* (2019), who did not find significant change in ALT and AST after phospholipid supplementation in lambs. The normal range of ALT and AST indicates that rice bran crude lecithin supplementation to calves had no harmful effect on liver, heart, other soft organs and skeletal muscles of the body. Cortisol mobilizes organism to fight stress, by affecting functioning of many organs and systems. It regulates nutrient partitioning through increasing lipolysis in adipose tissue (Larsen, 2013). The lower cortisol level in RBCL supplemented groups clearly shows that RBCL is helpful to reduce the stress of the body. Similar to the results of the present study, Ahmed *et al.* (2018) found no significant difference in plasma testosterone level after rumen protected fat supplementation in rams. Steroid hormones such as testosterone are biosynthesized from cholesterol and most steroidogenic cholesterol come from circulating lipoproteins (Miller, 2013) and feeding diet high in fat may increase the concentration of serum cholesterol and later increase serum steroid hormone.

CONCLUSION

It can be concluded that, RBCL can be incorporated at 12% in the concentrate mixture of crossbred calves replacing equal amount of maize grains

without any negative impact on plane of nutrition, clinical chemistry and performance of crossbred calves. Further studies in large number of livestock are warranted to explore the potential of RBCL at appropriate level in the ruminant ration.

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