Dietary Supplementation Of Rice Bran Crude Lecithin Affect Feed Digestion, Blood And Rumen Profile In Crossbred Calves

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Abstract

The present study was conducted to evaluate the effect of rice bran crude lecithin (RBCL) on nutrient digestion and balance, performance, methanogenesis, blood and rumen profile. Eighteen crossbred calves were randomly divided into three groups; RBCL-0, RBCL-8 and RBCL-12 and fed wheat straw based diet with concentrate mixture containing 0, 8 and 12 % RBCL respectively. The dry matter, organic matter and crude protein intake were comparable but tended to decrease with the RBCL levels. The digestibility of dry matter, organic matter, total carbohydrate and gross energy tended to decrease while crude protein and ether extract digestibility tended to increase with RBCL levels. The fibre fractions (NDF and ADF) digestibility was significantly ($P < 0.01$) lower in RBCL supplemented groups in comparison to control group. The body weight gain and average daily gain tended to decrease with increasing the level of rice bran crude lecithin. The percent of nitrogen and calcium retention tended to decrease, while phosphorus retention was significantly ($P < 0.01$) lower with inclusion level of RBCL. The methane production (L/d, L/kgW^{0.75}) were significantly ($P < 0.05$) lower in RBCL-12 followed by RBCL-8 as compared to RBCL-0 group. Serum biochemical did not show significant difference among dietary treatment groups. The cholesterol and blood urea concentration was significantly ($P < 0.01$) higher in RBCL-12 group as compared to control group. The rumen metabolites and microbiota showed reduction in RBCL-8 group as compared to RBCL-0 group without reach to significant ($P < 0.05$) level. It can be concluded, that detrimental effect of present levels (8 and 12%) of RBCL was seen in the performance of crossbred calves, which was associated with decreased fibre digestibility and fermentation in rumen. Beside this, RBCL is helpful in methane mitigation for cleaner production and can be a cheap source of energy in place of corn for ruminant. Further studies in large number of livestock are warranted to explore the potential of RBCL in the ruminant ration.

Introduction

Although India ranks first in milk production but the productivity of milk per animal is very less in rural areas. The main problem is the chronic shortage of protein (Digestible crude protein (DCP), 58%) and energy (Total digestible nutrients (TDN), 31%) rich animals feed. It is predictable that India will require 550 million tonnes of dry fodder, 105 million tonnes of concentrate and 1000 million tonnes of green fodder in year 2025 (Ravi Kiran et al., 2012). Due to high feedstuff prices and lacks of dietary grains, it is needed to explore the nonconventional agro-industrial byproducts in improving the availability of dietary energy sources for dairy animals. During refining of rice bran oil in the enzymatic degumming process, rice bran crude lecithin (RBCL) is produced as by product, roughly 1.5-2.0% of oil weight depending on the drying condition (Jala and Prasad, 2015). The current rice bran crude lecithin (RBCL) production in India is expected to fluctuate between 9000 and 12000 MT. In a number of countries, the gummy materials containing phospholipids are lost during degumming of crude rice bran oil, due to scarce literature (Senger et al., 2014). When using raw, standardized, liquid, non-fat and / or modified lecithin of various origins, fat feeding inhibition of rumen fermentation does not occur. Using lecithin instead of oil as the only lipid supplement was found to cause a lower ruminal degradation of crude protein due to their high

affinity to protein as a result of their amphiphatic properties which are not present in triglycerides and free fatty acids (Jenkins et al., 1989). Because they are amphiphatic (having hydrophobic and hydrophilic moieties), phospholipids are readily dispersed in aqueous solutions as micelles, giving surface active properties that enhance their wettability, fat dispersion and their affinity for protein. Therefore, when lecithin are used, attachment to feed particles or rumen microbes might be less pronounced and the release of the fatty acids could be delayed resulting in less adverse effects on rumen fermentation (Nagaraja et al., 1997). Sontakke et al. (2014b) observed no adverse effect of supplementation of rice bran lyso-phospholipids (RBLPL) on body weight, dry matter intake and milk production in lactating cows. Recently, several studies have attempted to apply lysophospholipid (LPL) to 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 animal performance to the dietary supplementation of LPL are inconsistent. Therefore present study is planned to investigate the effect of rice bran crude lecithin replacing corn on nutrient utilization, performance, methanogenesis and metabolic profile in crossbred calves.

Materials And Methods

Experimental animals and feeding

Eighteen crossbred male calves (*Bos taurus* x *Bos indicus*) (about 12 months old) were randomly divided into three groups (RBCL-0, RBCL-8 and RBCL-12) six animals each by completely randomized design. Prior to initiation of the experimental trial, animals were treated for ecto and endo-parasites as per the standard protocol. RBCL-0, RBCL-8 and RBCL-12 group calves were fed wheat straw based diet with three concentrate mixtures i.e. CM1, CM2 and CM3 for 120 days, respectively (Table 1). All the experimental calves were offered daily a weighed amount of concentrate mixture and wheat straw as per Kears (1985) feeding standards for 500 g daily body weight gain in the morning at 9:30 AM. The wheat straw was offered after the concentrate mixture was completely consumed by the animals. Fresh and clean drinking water was made available *ad libitum* twice a day. The body weight of calves was recorded at fortnightly intervals of experiment on the two consecutive days.

Table 1
Composition of feed and fodder fed to experimental calves

Ingredients	Concentrate mixtures			Wheat straw
	CM1	CM2	CM3	
Ingredient composition (%)				
Maize	42	34	30	
SBM	25	25	25	
Wheat bran	30	30	30	
Mineral mixture	2	2	2	
Salt	1	1	1	
RBCL	0	8	12	
Nutrient composition (%)				
DM	87.15 ± 0.47	87.64 ± 0.23	88.05 ± 0.11	89.62 ± 0.52
OM	94.20 ± 0.32	93.48 ± 0.05	93.40 ± 0.17	91.89 ± 0.08
CP	20.50 ± 0.15	20.04 ± 0.04	19.72 ± 0.15	3.46 ± 0.14
EE	3.02 ± 0.10	7.54 ± 0.15	10.25 ± 0.09	1.30 ± 0.02
TA	5.80 ± 0.32	6.52 ± 0.05	6.60 ± 0.17	8.11 ± 0.08
AIA	3.54 ± 0.17	3.78 ± 0.08	4.05 ± 0.09	6.12 ± 0.21
Total CHO	70.68 ± 0.58	65.90 ± 0.38	63.43 ± 0.73	87.13 ± 0.13
NDF	16.06 ± 0.58	15.76 ± 0.43	15.72 ± 0.40	70.30 ± 0.64
ADF	7.51 ± 0.40	7.37 ± 0.27	6.76 ± 0.43	48.47 ± 0.87
Hemicellulose	8.51 ± 0.29	8.52 ± 0.29	8.96 ± 0.43	21.83 ± 0.46
Ca	1.04 ± 0.02	1.07 ± 0.03	1.11 ± 0.03	0.38 ± 0.03
P	0.58 ± 0.01	0.72 ± 0.01	0.79 ± 0.01	0.10 ± 0.01
GE (kcal/kg)	4.26 ± 0.03	4.55 ± 0.02	4.88 ± 0.06	3.69 ± 0.05

Metabolism trial

A metabolism trial of 8 days duration including 2 days adaptation in metabolic cages followed by 6 days collection on all eighteen calves in specially designed cages, was conducted to determine nutrient

digestibility and balance after 60 days experimental feeding.

Indirect respiration chamber study

Following 90d of experimental feeding, animals were kept in respiration chamber for proper adaptation (2-3 days). The concentration of CH₄, CO₂ and O₂ in the chamber air was measured for three consecutive days using an infrared gas analyzer. The methane energy and heat production was calculated with the help of Brouwer's equation.

Metabolic profile

The blood from all calves' jugular vein was collected at morning during pre (0th d) and post (120th d) experiment. The serum was separated within 2 h after blood collection and analyzed promptly. Biochemical analysis was performed to determine the glucose, non-esterified free fatty acids (NEFA), total cholesterol, 3-hydroxy butyric acid (βHBA) levels to evaluate energy metabolism; total protein, serum albumin, globulin, A:G ratio and blood urea levels to evaluate protein metabolism; calcium and phosphorus level to evaluate mineral metabolism using commercial diagnostic kits as per manufacturer's recommendations. The growth related hormones *viz.* Insulin, IGF-1 and Leptin were analyzed by using commercial available ELISA diagnostic kits.

Rumen profile

Rumen liquor samples were collected at the 120th d of experimental feeding from RBCL-0 (non-supplemented) and RBCL-8 (supplemented) group animal, on the basis of growth trial results, by stomach tube method to observe the effect of RBCL on rumen fermentation and microbiota. Rumen metabolite mainly NH₃-N and VFA and its fractions were assessed. The NH₃-N concentration was analysed by using colorimetric assay as described in Weatherburn, (1967). Estimation of VFA concentration was done using a gas chromatograph equipped with a Flame Ionization Detector (Agarwal et al. 2008). Total bacteria, *Fibrobacter succinogenes*, *Ruminococcus albus*, *R. flavifaciens*, methanogens and fungi populations were assessed by qPCR (Real time PCR). In brief, the plasmid was extracted and serially diluted to make a standard curve and copy number was calculated (Ritalahti et al., 2006). The amplification reactions were performed in a total volume of 20 µl, containing 2 ng of template DNA, 10 µl of 2X Kappa SYBR master mix, 0.6 µl of each primer (10 µM) and nuclease free water (to make up the volume 20 µl). The number of protozoa was counted as per the procedure described by Kamra et al. (1991).

Chemical and statistical analysis

Analysis of offered feed, residual feed and faeces was done by standard procedure (AOAC, 2000). The gross energy of feed offered, faeces and urine was measured by ballistic bomb calorimeter (Gallenkamp), taking benzoic acid as standard. The data were statistical analyzed by statistical package SPSS version 20.0 in which data were subjected to ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means. Comparisons were made at 5% probability level.

Results

Nutrient Intake and digestibility

The chemical composition of feeds, daily nutrient intake and digestibility in experimental calves under dietary treatments (RBCL-0, RBCL-8 and RBCL-12) is presented in the Tables 1 & 2. The concentrate DM intake was tended to increase, while wheat straw DM tended to decrease with the inclusion level of RBCL. The DMI and OMI (g/KgW^{0.75}) was 1.5 and 8.5% lower in RBCL-8 and RBCL-12 groups compared to control group. The CPI (g/KgW^{0.75}) was comparable, while EEI (g/KgW^{0.75}) was significantly ($P < 0.001$) higher in RBCL-12 followed by RBCL-8 compared to RBCL-0 group. The TCHO intake (g/KgW^{0.75}) was significantly ($P < 0.01$) lower in RBCL-12 followed by RBCL-8 as compare to RBCL-0. The NDF and ADF intake was significantly ($P < 0.05$) lower in RBCL-12 group as compared to RBCL-0, while RBCL-8 group had intermediate position between RBCL-0 and RBCL-12 groups. The digestibility of DM, OM, TCHO and GE tended to decrease, while CP digestibility tended to increase with inclusion of RBCL levels. The EE digestibility was significantly ($P < 0.05$) higher in RBCL-12 group as compare to RBCL-0, however RBCL-8 had intermediate position between RBCL-0 and 12 groups. The NDF and ADF digestibility in RBCL-12 group were significantly ($P < 0.05$) lower than RBCL-0 followed by RBCL-8 group.

Table 2. Effect of various levels of RBCL on nutrient intake and their digestibility and growth performance in crossbred calves

^{abc}Means with different superscripts within a row differ significantly

Nutrient Utilization

Nitrogen and Energy balance

The results obtained related to nitrogen and energy balance under dietary treatment groups in crossbred calves are presented in the Table 3. Total N intake, faecal N, urinary N and N retention (g/d) was comparable among dietary treatment groups. The RBCL-12 group had 1 % lower faecal N (% of N excretion) and 2.5 % higher urinary N (% of N excretion) than control, while RBCL-8 had similar faecal N

Attributes	Dietary treatments			SEM	P value	(% of N excretion) and 1.5 % higher urinary N (% of N excretion) than RBCL-0 group. The GE, DE and ME intake (Mcal/d) in RBCL-0, RBCL-8 and RBCL-12 groups were comparable but numerically higher in RBCL-8 and lower in RBCL-12 group. The FE (%) was tended to increase, while UE (%) tended to decrease with the increasing level of RBCL. The methane energy (%) was significantly (P < 0.01) lower in RBCL-12 followed by RBCL-8 as compared to RBCL-0 group. The energy utilization (DE:GE, ME:GE and ME:DE) was comparable among dietary groups
	RBCL-0	RBCL-8	RBCL-12			
Metabolic Size (kgW ^{0.75})	49.97	49.02	47.70	1.31	0.804	
Feed DM intake (kg/d)						
Concentrate	2.64	2.74	2.76	0.04	0.634	
Roughage	1.74	1.51	1.07	0.12	0.061	
Nutrient intake						
DM (g/kg W ^{0.75})	87.77	86.56	80.32	1.63	0.128	
OM (g/kg W ^{0.75})	81.88	80.44	74.69	1.51	0.116	
CP (g/kg W ^{0.75})	12.12	12.41	12.29	0.27	0.923	
EE (g/kg W ^{0.75})	2.07 ^c	4.64 ^b	6.29 ^a	0.41	0.001	
TCHO (g/kg W ^{0.75})	67.66 ^a	63.45 ^a	56.10 ^b	1.70	0.008	
NDF (g/kg W ^{0.75})	32.79 ^a	30.21 ^{ab}	24.55 ^b	1.40	0.029	
ADF (g/kg W ^{0.75})	20.71 ^a	18.86 ^a	14.54 ^b	1.01	0.021	
Nutrient digestibility (%)						
DM	63.24	62.04	61.96	0.89	0.296	
OM	65.87	64.20	64.02	0.90	0.674	
CP	73.62	74.15	74.62	0.83	0.904	
EE	81.85 ^b	83.36 ^{ab}	85.42 ^a	0.59	0.047	
TCHO	63.27	60.57	59.48	1.02	0.291	
NDF	53.06 ^a	47.38 ^a	38.59 ^b	1.98	0.003	
ADF	48.31 ^a	40.86 ^a	28.94 ^b	2.57	0.001	
Hemicellulose	60.97	58.15	58.29	1.58	0.745	
GE	59.44	58.54	57.24	1.09	0.741	

Table 3. Effect of various levels of RBCL on nitrogen and energy balance in crossbred calves

Attributes	Dietary treatments			SEM	P value
	RBCL-0	RBCL-8	RBCL-12		
Nitrogen balance					
N intake and excretion (g/d)					
N intake	96.30	96.32	93.00	1.71	0.576
N faeces	25.47	25.12	23.52	0.98	0.711
N urine	29.83	30.14	31.60	0.94	0.807
N retention	41.00	40.26	38.37	1.71	0.831
% of N excretion					
Faeces	26.39	26.21	25.35	1.02	0.916
Urine	30.83	32.37	33.52	1.15	0.461
% N retention	42.78	41.42	41.13	1.69	0.900
Energy balance					
Energy intake (Mcal/d)					
Gross energy (GE)	17.78	18.03	17.40	0.52	0.843
Digestible energy (DE)	10.60	10.56	10.07	0.34	0.640
Metabolizable energy (ME)	9.23	9.41	9.05	0.32	0.810
Energy loss (Mcal/d)					
Faecal energy	7.18	7.47	7.33	0.30	0.932
Urinary energy	0.28	0.30	0.33	0.02	0.497
Methane energy	1.09 ^a	0.86 ^b	0.70 ^c	0.05	0.001
Retained energy	6.57	6.74	6.63	0.28	0.924
Energy loss in percent (%)					
Faecal energy	40.36	41.46	42.76	1.09	0.741
Urinary energy	1.60	1.64	1.87	0.13	0.624
Methane energy	6.14 ^a	4.80 ^b	4.05 ^b	0.25	0.001
Retained energy	36.82	37.30	38.10	1.13	0.865
Energy utilization (Mcal/Mcal)					
DE/GE	0.60	0.59	0.58	0.01	0.238

ME/GE	0.52	0.52	0.52	0.01	0.451
ME/DE	0.87	0.89	0.90	0.01	0.276

^{abc}Means with different superscripts within a row differ significantly

Calcium and Phosphorus balance

The calcium and phosphorus balance in calves is shown in Table 4. The Ca intake (g/d) was comparable, while faecal calcium (g/d, %) tended to increase with inclusion level of RBCL. The urinary Ca and Ca retention (g/d, %) tended to decrease with the level of RBCL. The P intake (g/d) was significantly ($P < 0.01$) higher in RBCL-12 and RBCL-8 as compare to RBCL-0. The faecal P (g/d, %) was comparable among the RBCL-0, RBCL-8 and RBCL-12, however urinary P (%) was tended to decrease with RBCL levels. The phosphorus retention was significantly ($P > 0.010$) lower in RBCL-12 and RBCL-8 as compared to RBCL-0 group.

Table 4
Effect of various levels of RBCL on mineral balance in crossbred calves

Attributes	Dietary treatments			SEM	P value
	RBCL-0	RBCL-8	RBCL-12		
Calcium balance					
Ca intake and excretion (g/d)					
Ca intake	34.11	35.07	34.66	0.76	0.883
Ca faeces	16.66	18.47	19.62	0.52	0.062
Ca urine	4.95	4.22	3.43	0.27	0.074
Ca retention	12.50	12.37	11.61	0.60	0.693
% of Ca excretion					
Faeces	48.74	52.83	57.12	1.15	0.001
Urine	14.64	12.39	10.05	0.94	0.146
% Ca retention	36.62	34.79	33.36	1.19	0.471
Phosphorus balance					
P intake and excretion (g/d)					
P intake	17.08 ^b	21.25 ^a	22.85 ^a	0.68	0.000
P faeces	10.54 ^b	14.79 ^a	16.44 ^a	0.76	0.000
P urine	0.06	0.06	0.06	0.01	0.754
P retention	6.47	6.41	6.36	0.25	0.641
% of P excretion					
Faeces	61.87 ^b	69.50 ^a	72.03 ^a	1.78	0.007
Urine	0.35	0.29	0.25	0.03	0.162
% P retention	37.78 ^a	30.21 ^b	27.72 ^b	1.78	0.008
^{ab} Means with different superscripts within a row differ significantly					

Growth Performance And Methanogenesis

Effect of various dietary levels of RBCL on growth performance and methane production and heat production is presented in Table 5. Initial and final body weights were comparable among the groups. The total BW gain and ADG tended to decrease with the level of RBCL in the ration of calves. The final body weight of RBCL-8 and RBCL-12 group calves was 3 and 6% lower than RBCL-0 group calves. During respiration chamber study, the metabolic body size was comparable among dietary treatments. The DMI (kg) depression was 13 and 17.5 % in RBCL-8 & RBCL-12 groups in comparison to control. The respiratory quotient (RQ) of RBCL-0, RBCL-8 & RBCL-12 groups were 1.06, and 1.04 and 1.04, respectively and comparable. The methane production (L/d, L/kgW^{0.75}) were significantly ($P < 0.05$) lower in RBCL-12 followed by RBCL-8 as compared to control group. There was 9 and 21% reduction in methane production (L/kg DMI and L/kg OMI) in RBCL-8 and RBCL-12 groups in comparison to control (RBCL-0) group. Total heat production (Mcal/d, Kcal/d/W^{0.75}) of RBCL-8 and RBCL-12 group was 2 and 11% lower than RBCL-0 group. The total methane energy loss (Kcal/d/W^{0.75}) was significantly ($P < 0.05$) lower in RBCL-8 and RBCL-12 groups as compared to control (RBCL-0) group with 19 and 33% reduction.

Table 5
Effect of various dietary levels of RBCL on growth performance, methane and heat production in crossbred calves

Attributes	Dietary treatments			SEM	P value
	RBCL-0	RBCL-8	RBCL-12		
Body weight (kg)					
Initial	128.67	126.83	126.67	4.66	0.983
Final	227.50	220.33	214.67	6.40	0.739
Total Gain	98.83	93.50	88.17	2.70	0.289
ADG (g)	823.67	779.17	734.33	22.47	0.283
Respiration chamber study					
Metabolic size (kgW ^{0.75})	59.52	57.74	56.81	1.28	0.711
DMI (kg)	5.03	4.35	4.15	0.19	0.156
CO ₂ produced (L/d)	1327.04	1283.14	1160.42	56.17	0.469
O ₂ consumed (L/d)	1255.64	1234.48	1109.88	52.17	0.481
RQ	1.06	1.04	1.04	0.01	0.621
CH ₄ production (L/d)	114.88 ^a	90.68 ^b	73.04 ^c	5.41	0.001
CH ₄ production (L/kgW ^{0.75})	1.94 ^a	1.57 ^b	1.29 ^b	0.09	0.001
CH ₄ production (L/kg DMI)	23.08	21.06	18.10	0.98	0.095
CH ₄ production (L/kg OMI)	24.82	22.89	19.68	1.04	0.111
HP (kcal/d/W ^{0.75})	110.34	106.32	97.48	5.25	0.741
CH ₄ energy loss (kcal/d/W ^{0.75})	18.49 ^a	14.95 ^b	12.26 ^b	0.82	0.001
^{ab} Means with different superscripts within a row differ significantly					

Blood profile

The data of serum biochemical related to protein, energy and mineral metabolism and serum hormones is presented in Table 6. The treatment mean of serum total protein, albumin, globulin (g/dl) and A:G ratio

was comparable, while period mean of serum total protein, albumin (g/dl) and A:G ratio was significantly ($P<0.01$) higher at 120 d compare to 0 d.. The treatment mean of blood urea (mg/dl) was significantly ($P<0.01$) higher in RBCL-12 group than RBCL-8 and RBCL-0 groups while period mean significantly higher at 120 d than 0 d. The treatment mean of serum glucose (mg/dl) was comparable among groups, while period mean tended to increase with time. The treatment mean of NEFA ($\mu\text{mol/L}$) was numerically lower in supplemented groups as compare to control group, while period mean was also quantitatively lower at 120 d as compare to 0 d. The treatment mean of cholesterol (mg/dl) was significantly ($P<0.001$) lower in RBCL-12 group as compare to RBCL-0 and 8 groups, while period mean was significantly ($P<0.01$) lower in 120 d than 0 d. The treatment mean of BHBA (nmol/ml) was numerically lower in lecithin groups than control while period mean was significantly ($P<0.001$) lower in 120 d than 0 d. The treatment and period mean of serum Ca was comparable among groups and period, while treatment mean of serum i-P was comparable between groups and period mean was significantly higher at 120 d than 0 d. The treatment mean of serum insulin ($\mu\text{IU/ml}$) were comparable among groups and tended to decrease with the higher level of RBCL, while period mean were significantly ($P<0.05$) higher at 120d as compared to 0d. The treatment mean of IGF-1 (ng/ml) of RBCL-0, RBCL-8 and RBCL-12 groups was analogous to each other but tended to decrease. The period mean of IGF-1 was numerically higher but not reached to significant ($P<0.05$) level. There was no significant difference in Insulin and IGF-1 among dietary groups due to period treatment interaction. The treatment mean of serum leptin (ng/ml) were comparable among groups, while period mean were significantly ($P<0.05$) higher at 120d as compared to 0d.

Rumen profile

Among three dietary groups, rumen metabolites and microbial profile were studied in non-supplemented (RBCL-0) and supplemented (RBCL-8) groups on the performance basis. The supplementation of RBCL in RBCL-8 group did not cause significant ($P<0.05$) change in the concentration of $\text{NH}_3\text{-N}$, total VFA and its fraction i.e. acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate, A:P and (A+B):P ratio in comparison to RBCL-0 calves. The concentration of all above rumen metabolites was lower in RBCL supplemented group. The population density of rumen microbes including total bacteria, fungi, methanogens, ciliate protozoa, fibrolytic bacteria as *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavifaciens* of crossbred calves was comparable in both groups (Table 7). In RBCL-8 group, numerically lower count of total bacteria, fibrolytic bacteria, fungi, archaea and protozoa was seen as compare to control group.

Discussion

Nutrient intake and digestibility

The nutrient intake and digestibility of our study are compatible with Lee et al. (2019), Shain et al. (1993) and Jenkins et al. (1989), who found that increasing lysophospholipid tended to decrease intake and apparent digestibility of DM, OM and NDF. Sontakke et al. (2014b) observed no significant difference in

nutrients intake and digestibility, however, significantly higher EE digestibility due to RBLPL inclusion. Contrary to present findings, Huo et al. (2019) found that LPL supplementation increased DM and CP digestibility but large decrease in NDF and ADF digestibility. The RBCL contains higher amount of unsaturated fatty acids and it is well established that unsaturated fatty acids are toxic for rumen microbes therefore decrease the fibre digestion. Sabiha (2009) stated that the lysophospholipids (lecithin) enhance the nutrient digestion and absorption by their ability to form comparatively smaller micelles and by increasing the flux rate of different digested nutrients across the cell membrane by improving permeability. Wettstein et al. (2000a) noticed that raw canola lecithin and deoiled soy lecithin improved the apparent digestibilities of dry matter and organic matter. Yoon et al. (1986) found an increase in fibre digestibility in sheep with the addition of 3.4% soy lecithin/kg total dry matter. Abel-Caines et al. (1998) found a higher NDF digestibility with a mixture of soy lecithin, soybean soapstock and soy hay (SLSSSH). The inconsistent results of nutrient intake and digestibility may be due to the duration of experiments, source of lecithin, method of manufacturing, dose, degradation in the rumen and rumen bypass of lecithin. Zampiga et al. (2016) and Polycarpo et al. (2016) reported that LPL supplementation on nonruminant animals increased of apparent nutrient digestibility, thereby improving feed efficiency.

Nitrogen and Energy balance

Wettstein et al. (2000a) reported that at a similar nitrogen intake, faecal nitrogen voided was numerically lowest with the raw canola lecithin diet and overall nitrogen balance was positive in all diets. It has been well established that the N utilization efficiency can be improved through synchronously supply of adequate fermentable energy and N for maximum microbial growth in the rumen and capture ammonia for protein synthesis (Dijkstra et al., 2011). The nitrogen utilization efficiency is not improved due to inadequate fermentable energy for rumen microbes in present experiment, which supports the reduction in ADG. The metabolizability values obtained in this study were 0.51-0.52 and these were in the normal range (0.40-0.64) proposed in several reports (ARC, 1980; Kamalzade et al., 2004). The ME:DE values were above the generalized value 0.82, suggested by Blaxter (1962) and ARC (1965). The results of present findings are in conformity with Huo et al. (2019), suggested that the status of nitrogen and energy in the body has not been improved, which supports the outcome of no or a slight difference in ADG. Contrary to present finding, Lee et al. (2019) found decrease in urinary N excretion with increasing LPL in diet, however Shain et al. (1993) reported that cows fed the high SLS diet had the highest positive energy balance. The lower methane energy loss in rice bran supplemented groups could be related to lower methane production due to either bio-hydrogenation of unsaturated fatty acids or low acetate production in the rumen. The low HI is associated with lower DMI along with the higher level of RBCL in ration of experimental calves. The net energy available for growth and maintenance is only 5 % higher in RBCL-4 group and equal in RBCL-6 group in comparison to RBCL-0 group.

Ca and P balance

Because of limited information about lecithin in ruminants, studies with nonruminant animals fed lecithin is discussed. In the support, Overland et al. (1994) noticed that lecithin had no impact on the retention of total P and Ca in pigs while contrary Huang et al. (2007) observed that lecithin at 2% level significantly improved the Ca and P utilization in broilers. In current study, the higher faecal Ca might be attributed to the formation of insoluble calcium soap from by reaction of Ca with free long chain fatty acids (LCFAs) present in RBCL. While RBCL is a good source of phosphorus but the phosphorus absorption is inversely proportional to the intake, might be possible explanation behind less phosphorus retention. The lower Ca and P retention is also correlated with the reduction in ADG in experimental calves.

Growth performance and methanogenesis

In the conformity of present findings, Shain et al. (1993) reported that daily gain and feed efficiency were not affected by SLS replaced corn grain in beef calves. Lee et al. (2019) stated that any improvements in animal performance by LPL, if existent, would come from increased utilization efficiency of feed ingested rather than more nutrients supplied. Contrary to our results, Huo et al. (2019) and Li et al. (2016) reported increased ADG in LPL treatment but difference did not reach the level of significance. The inconsistent results of animal performance may be due to the trial period, , method of manufacturing, dose, and source of lecithin, degradation in the rumen and rumen bypass of lecithin. Lysophospholipids as a feed additive have been examined mostly with nonruminant animals, where increased growth rates and feed efficiency have been observed by feeding LPL to chicken and pigs (Zhao et al., 2015, 2017; Zampiga et al., 2016).

In the concurrence of present findings, Sontakke et al. (2014a) and Wettstein et al. (2000b) reported lower methane production with supplementation of RBLPL and soy lecithin. The results are also in agreement with Benchaar et al. (2001), Hart et al. (2009), Yan et al. (2010) and Lima et al. (2013), who reported a decrease in CH₄ production (kcal/d) with decreased DMI in cattle. PUFA also has an inhibitory effect on methane production through direct use of hydrogen by saturation in the rumen (Rasmussen and Harrison, 2011). Inhibitory effects of unsaturated fatty acids can be expected for methanogens and, maybe to a lesser degree, for Gram-positive cellulolytic bacteria (Nagaraja et al., 1997) and ciliates (McAllister et al., 1996) which provide hydrogen as a substrate for the methanogens. The lower reduction of methane release with lecithin than with canola oil indicates that phospholipids were either not hydrolysed to the same extent or slower as triglycerides (Jenkins et al., 1989). In current study the lower methane production may be attributed to lower DMI and presence of PUFAs.

Metabolic profile

In present study, the higher serum urea and cholesterol level in RBCL-12 group may be attributed to the insufficient utilization of ammonia by ruminal microbes due to lower soluble carbohydrate and supplementation of higher amount of fat in the diet. Plasma glucose, NEFA and BHBA are considered as principle circulating blood metabolite to assess the energy status of the animals (Muwel, 2016). The positive balance of Ca and i-P is clearly reflected by the serum levels. The results of our study are in

conformity with the finding of Li et al. (2016), who observed that soy lecithin in the diet did not affect concentrations of serum glucose, albumin, total protein and calcium while increased the serum concentration of triglyceride, total cholesterol and HDL-cholesterol in steers. Huo et al. (2019) found that glucose, total protein, albumin, globulin, blood urea and total cholesterol did not alter with the supplementation of LPL in lambs. Lough et al. (1991) showed that feeding soy lecithin to lambs increased cholesterol. Jenkins (1990) reported lecithin had no effects on plasma non-esterified fatty acids and glucose concentrations. Contrary, Jenkins et al. (1989) observed lecithin increased serum NEFA but had no effect on concentrations of glucose, triglyceride or total cholesterol. The results of our study is in the compatible with the Yildiz et al. (2003) and Cha and Jone (1998), who found that fat feeding increases plasma leptin concentration, however, Becú-Villalobos et al. (2007) found lower leptin level in fat supplementation. Researches demonstrated that the plane of nutrition directly influences the circulating level of IGF-1 and IGF-1 along with insulin are reported to indicate the nutritional status of the animals (Ciccioli et al., 2003; Lents et al., 2005). It has been well established that both dietary energy and protein are the principal nutritional determinant for basal circulating plasma concentration of IGF-1 (Elsasser et al., 1989).

Rumen profile

In the accordance of present study, Sontakke et al. (2014a) observed that the production of acetate, propionate and butyrate was similar upto 10% level of RBLPL inclusion in the ration. Wettstein et al. (2000b) reported that lecithin increased propionate proportion along with total VFA concentration but reduced the apparent ruminal protein degradation. Bacteria counts were reduced by the inhibitory effect of fatty acids on fibre degrading bacteria (McAllister et al., 1996) and also by the generally lower supply of fermentable matter because a part of the carbohydrates was replaced by lipids. On the other hand, ciliates numbers were higher in all lecithin diets with increasing dispersibility in water which may be the reason for the lower bacteria counts found with the lecithins (Jouany and Ushida, 1999). Kim et al. (2020) observed that LPL supplementation increased the proportion of butyrate, valerate, and iso-valerate but tended to decrease propionate. Considering that glycerol which is one of the main products in lipid metabolism could be fermented into propionate, butyrate, valerate, and iso-valerate rather than acetate. Total bacteria increased in a linear manner. Ciliate protozoa unaffected but fungi, *F. succinogenes* and *R. albus* were significantly decreased in a linear manner by LPL supplementation. Huo et al. (2019) found 37% higher ammonia-N concentration and 61% higher total SCFA concentration in lecithin group in lambs. Lee et al. (2019) observed reduced proportion of acetate in total VFA with no differences in propionate proportion, resulting in a tendency for decreasing the ratio of acetate to propionate. Contrary, Cho et al. (2013) noticed that ammonia nitrogen (ammonia-N), TVFA, acetate, propionate, butyrate and valerate production increased in LPLs treatments compared with control for whole incubation times, however, A:P ratio decreased. Abel–Cains (1996) found similar ratio of acetate to propionate, while higher total, cellulolytic, carboxy-methylcellulose degrading bacterial and protozoal counts in TMR containing soy lecithin, soy soap stock and soy hay (SLSSSH) compare to control. Yoon et al. (1986) also observed no change in butyrate proportion on 8% lecithin supplementation in the diet of sheep. Jenkins et al. (1989) reported decreased acetate and increased propionate proportions but the proportion of butyrate

was not influenced by phospholipids in sheep. Jenkins (1990) found a decrease in acetate proportion and increased butyrate when lecithin was added in combination with hydrogenated fat to steer diet. Paul (1994) observed a decrease in butyrate production during in-vitro study when pure phospholipids were used in comparison to free fatty acid and triglycerides.

Conclusion

In last it can be concluded that excessive levels of RBCL (8 and 12 %) did not adversely affect the nutrients intake, digestibility, metabolic profile and growth performance of crossbred calves, except the fibre digestibility and its intake. RBCL significantly reduce the methane production so it is helpful in methane mitigation for cleaner production and can be a cheap source of energy in place of corn for ruminant. Further studies in large number of livestock are warranted to explore the potential of RBCL at appropriate level in the ruminant ration. Mechanism studies are needed to explore the effect of RBCL on rumen fermentation and body metabolism.

Declarations

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Conflict of interest:

The authors declare no competing interest.

Ethics approval

This study was approved by CPCSEA ethical committee, Ministry of Fisheries, Animal Husbandry and Dairying, New Delhi, for animal experimentation according to this reference No. 25/2/2020-CPCSEA-DADF, dated 13 April,2020.

Consent of participate

The consent was obtained from all individual participants included in this study.

Consent of publication

All authors agreed to have the findings of this research published.

Availability of data

The datasets used during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Author's contribution

V.B. Chaturvedi, L.C. Chaudhary and A.K. Verma conceived and design the research. Dharmesh Tewari conducted the animal experimentation and lab work and wrote original draft. S.K. Chaudhary helped in the statistical analysis of data. All author read and approved the final manuscript.

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