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Effect of Rice Bran Crude Lecithin Supplementation on Feed Intake, Nutrient Utilization, and Methane Emission in Crossbred Cattle

P. Bagavan Reddy, V.B. Chaturvedi*, L. C. Chaudhary, Anju Kala and P. Thamizhan
Centre of Advanced Faculty Training in Animal Nutrition,
ICAR-Indian Veterinary Research Institute, Izatnagar-243122 UP, India
*Correspondence: chaturvedivb@gmail.com

ABSTRACT

An experiment was conducted to ascertain the level of inclusion of Rice Bran Crude Lecithin (RBCL) and its effect of supplementation on voluntary feed intake, nutrient utilization, and methane emission in adult crossbred cattle. Eighteen adult crossbred cattle with an average body weight (BW) of 315.6±19.95 kg were selected and randomly divided into three groups (n=6), namely, RBCL-0, RBCL-4, and RBCL-8 by Randomized Block Design (RBD). An experimental diet was offered to the animals in the form of a total mixed ration (TMR) containing wheat straw and concentrate mixture in the proportion of 70:30 in restricted feeding mode. The RBCL was added at the rate of 0, 4, and 8% in the concentrate mixture of RBCL-0, RBCL-4, and RBCL-8 groups respectively by replacing the equal amount of corn. $CaCO_3$ was added in the concentrate mixture at the rate of 0.1, 0.15, and 0.3% in RBCL-0, RBCL-4, and RBCL-8 groups respectively, to maintain the Ca and P ratio. The feeding trial was conducted for a period of 120 days. The dry matter intake(DMI) and BW changes were recorded fortnightly. The voluntary feed intake of the experimental animals was found similar (P>0.05) among the treatment groups. The intake and digestibility of DM, OM, CP, TCHO, NDF, and ADF were not affected by the RBCL supplementation, whereas the intake and digestibility of EE were significantly (P<0.01) higher in RBCL-supplemented groups. The dietary supplementation of RBCL did not influence nitrogen balance, energy partitioning, and methane emissions in experimental animals. The present research findings showed the potential of RBCL as an energy source in a ruminant diet without showing any adverse effects on voluntary feed intake and nutrient utilization.

KEYWORDS: Methane emission, Nutrient utilization, Rice bran crude lecithin, TMR

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INTRODUCTION

India has the largest livestock population in the world with nearly 536 million animals (Livestock Census, 2019), making our country the largest milk producer in the world and contributing 23% to global milk production. However, due to the constant shortage of feed, quality feeding of livestock is a major problem in our country. At present, our country has a net deficit of 35.6% green fodder, 44% concentrate ingredients, and 10.95% dry fodder (IGFRI, 2015). In the coming years, feed demand may increase further due to a steady increase in the livestock population. In this scenario, efficient livestock production can only be achieved by providing suitable feed and forage resources (Makkar and Ankers, 2014) and efficient utilization of available crop residues and agro-industrial by-products in the preparation of complete feeds for livestock (Bhargava et al., 2022).

Rice bran crude lecithin (RBCL) is one such agro-industrial by-product generated during the refining of rice bran oil. The use of rice bran lecithin/lysophospholipids (LPL) as a feed additive in the diets of monogastric animals has been a common practice due to its ability to improve nutrient digestibility, growth rate, and feed efficiency (Zhao et al., 2015; Zampiga et al., 2016; Wang et al., 2019). However, its use in ruminant diets is limited because it has been assumed to have negative effects on rumen fermentation like typical crude fat/oil additives. However, some of the available studies have shown the potential benefits of lecithin incorporation in ruminant diets (Jenkins and Fotouhi, 1990; Rico et al., 2017; Lee et al., 2019) in terms of its emulsifying properties and improved digestibility and absorption of dietary fat and fat-soluble vitamins, and some of the *in vitro* and *in vivo* studies have shown that the addition of lecithin can also reduce methane emission in ruminants (Wettstein et al., 2000a; Sontakke et al., 2014a; Tewari et al., 2021). With this in mind, the current study was conducted to investigate the effects of RBCL supplementation on feed intake, nutrient utilization, and methane emissions in adult crossbred cattle.

MATERIALS AND METHODS

The present experimental procedure was approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-IVRI, Izatnagar, as per the guidelines of the CPCSEA, New Delhi for animal experimentation.

Experimental design, animal housing and feeding management

This experiment was done at the Experimental Animal Shed, Animal Nutrition Division, ICAR-IVRI, Izatnagar (UP). For this study, eighteen (18) adult male crossbred cattle (Average BW 315.66±19.95 kg; Age 30-36 months) were selected and randomly divided into three groups namely, RBCL-0, RBCL-4, and RBCL-8, based on their body weight. Prior to the feeding trial, all the experimental animals underwent deworming for internal and external parasites. The experimental animals were fed with TMR containing wheat straw and concentrate mixture in 70:30 proportion in restricted feeding mode to meet their nutrient requirements as per the ICAR (2013) for a period of 120 days. RBCLwas added at the rate of 0, 4, and 8% in the concentrate mixture by replacing the equal quantity of maize in RBCL-0, RBCL-4, and RBCL-8 respectively (Table 1).Calcium carbonate ($CaCO_3$) was added at the rate of 0.1, 0.15, and 0.3% in the concentrate mixture of RBCL-0, RBCL-4, and RBCL-8 groups respectively, to maintain the Ca: P balance.The experimental animals were offered a daily weighed amount of dietary TMR and also provided with Napier Bajra green fodder (0.5kg/animal) once a week to meet their vitamin A requirement. Clean and fresh drinking water was made available in the morning (9:00 AM) and evening (16:00 PM) to all the experimental animals throughout the feeding trial.

Metabolism trial

A metabolism trial was conducted after 60 days of the feeding trial,during which five animals from each group were selected randomly and kept in specially designed metabolic cages, individually. The animals were weighed before and after the metabolism trial to get an average body weight. After the proper adaptation period, feed offered, residue left, faeces and urine voided were collected for 6 days, to determine the different nutrient digestibility and balance of N, Ca, and P.

Table 1. Ingredients and chemical composition (%) of dietary TMR used in the experiment (% DM basis)

Ingredient composition (%)	TMR†		
	RBCL-0	RBCL-4	RBCL-8
Wheat straw	30	30	30
Concentrate	70	70	70
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
RBCL	0	4	8
$CaCO_3$	0.1	0.15	0.3
Chemical composition (% DM)			
OM	92.8	92.7	92.4
CP	7.97	7.95	7.88
EE	1.65	2.21	4.68
TA	7.14	7.27	7.63
TCHO	83.2	82.6	79.8
NDF	66.0	65.6	64.6
ADF	38.9	37.6	36.2
Hemicellulose	27.1	27.9	28.4
Ca	1.13	1.21	1.35
P	0.55	0.67	0.82
Ca: P	2.05	1.81	1.65
GE (kcal/g)	4.44	4.57	4.94

† Total mixed rations without (RBCL-0) or with rice bran crude lecithin (RBCL-4 and RBCL-8)

Indirect respiration calorimeter study

During the feeding trial, four animals from each group were selected and kept inside the open circuit respiration chamber one after the other with a proper adaptation period of two days to estimate the enteric methane (CH_4) and carbon dioxide (CO_2). Animals were provided with a daily weighed amount of TMR and fresh, clean drinking water inside the respiration chamber. The wet bulb and dry bulb temperature, flow rate, volume of the gases, and atmospheric pressure were noted at hourly intervals. During the chamber study, the concentration of CH_4,CO_2, and O_2 in the chamber air was measured for two consecutive daysby using the infrared gas analyser. The heat production (HP) and methane emissions were calculated by using Brouwer's equation (Brouwer, 1957).

$$HP\ (kcal/d) = 3.866\ O_2 + 1.200\ CO_2 - 0.518\ CH_4 - 1.431\ N_2$$

Where, O_2 = Volume of Oxygen consumed (L/d); CO_2= Volume of Carbon dioxide produced (L/d); CH_4= Volume of Methane produced (L/d); N_2= Amount of nitrogen excreted in urine (g/d)

Chemical analysis

The samples collected during the metabolism trial including representative samples of feed offered, residue, and faeces were analyzed for proximate principles as per the standard protocol given by AOAC(2000) and fibre fractions as per the standard protocol given by Van Soest et al. (1991). The gross energy (GE) of feed offered, residue, faeces, and urine were estimated by using the ballistic bomb calorimeter with benzoic acid as standard.Calcium (Ca) and Phosphorus (P) content in the feed offered, faeces, and urine voided were estimated as per the standard method given by Talapatra et al. (1940) and AOAC (2000) respectively.

Statistical analysis

The data generated from different analytical procedures was analyzed statistically (Snedecor and Cochran, 1994) by using the SPSS version 20.0 package to determine the statistical significance difference ($P<0.05$) among the dietary treatment groups.

RESULTS AND DISCUSSION

Feed intake and nutrient utilization

The chemical composition of the dietary TMR fed to the experimental animals was given in Table1.The average daily DM intake of experimental groups supplemented with dietary TMR containing RBCL (RBCL-4 & RBCL-8) was comparable to the control group (RBCL-0) without showing any significant difference ($P>0.05$) (Table 2). Similar kind of results has been reported by Jenkins, (1990) on supplementation of lecithin in a hydrogenated fat-containing diet in the steers. Similarly, Sontakke et al. (2014b) also did not observe any significant effect on DMI on the supplementation of rice bran lysophospholipids up to 6% level in the diet of crossbred lactating cows.Huo et al. (2019) also found no effect on DMI on the supplementation of lysophospholipids (LPL) in the diet of lambs. In contrast to our study, Tewari et al. (2022) observed a reduced roughage DMI (kg/d) ingrowing crossbred calves supplemented with12% RBCL in the diet.Fontoura et al. (2021) also reported a similar decrease in DMI with the increased level of deoiled soy lecithin in the cows fed with a palm fatty acid-based diet. The decreased DMI in some of thesestudies might be attributed to the higher level of dietary fat used in the experiment which may reduce the digestibility of DM and fibre by negatively affecting the rumen microbial population and altering the rumen fermentation. However, in our present study, the level of lecithin does not influence DMI as the overall fat level in the diet was within the recommended level. The intake of other nutrients OM, CP, TCHO, NDF, and ADF (g/kg $W^{0.75}$) were comparable among the treatment groups without showing any significant difference ($P>0.05$), whereas EE intake (g/kg $W^{0.75}$) was significantly higher ($P<0.05$) in RBCL-8 group followed by RBCL-4 group than the RBCL-0 group, which is due to the addition of graded levels of RBCL among the groups.

Table 2. Effect of feeding dietary TMR containing RBCL on nutrient intake and digestibility in crossbred cattle

Attributes	dietary groups			SEM	P value
	RBCL-0	RBCL-4	RBCL-8		
Body weight (kg)	328.8	342.2	329.3	11.23	0.876
Metabolic Size (kg $W^{0.75}$)	77.1	79.4	77.2	1.98	0.879
DMI (kg/d)	5.97	5.95	5.89	0.17	0.996
DMI (% BW)	1.82	1.74	1.79	0.03	0.457
Nutrient intake (g/kg $W^{0.75}$)					
DM	78.2	75.1	77.3	1.12	0.550
OM	70.8	73.7	71.3	1.83	0.819
CP	5.45	5.22	5.32	0.08	0.521
EE	1.29^{a}	1.66^{b}	3.62^{c}	0.27	<0.001
TCHO	66.7	63.6	63.4	0.99	0.322
NDF	59.4	56.8	57.7	0.86	0.470
ADF	38.2	35.8	35.7	0.60	0.148
Nutrient digestibility (%)					
DM	55.4	54.1	53.9	1.02	0.682
OM	56.8	56.4	55.9	0.96	0.058
CP	62.6	63.8	64.2	0.89	0.764
EE	81.0^{a}	85.5^{ab}	89.6^{b}	1.30	0.013
TCHO	62.8	62.5	58.2	1.05	0.133
NDF	52.1	51.2	50.6	0.53	0.523
ADF	47.4	46.8	46.2	1.38	0.950
GE	56.0	56.2	54.1	1.08	0.688

[abc]Means within the row with different superscripts differ significantly ($P<0.05$)

The supplementation of RBCL did not show any adverse effect on the digestibility of DM, OM, CP, TCHO, NDF, ADF, and GE and were found similar among the dietary treatment groups. However, the digestibility of EE was significantly (P<0.05) higher in the RBCL-8 group followed by the RBCL-4 group compared to the RBCL-0 group. A similar, improvement in EE digestibility has been observed by Jenkins et al. (1989), on supplementation of the deoiled and crude soybean lecithin (5.2%) in the diet of lambs compared to that of the corn oil diet. Similarly, a higher EE digestibility was observed on supplementation of rice bran phospholipids (6%) in crossbred cows (Sontakke et al., 2014b). Tewari et al. (2022) also observed a similar improvement in EE digestibility on feeding RBCL to crossbred calves. The possible reason for increased EE digestibility in the present study was might be due to the emulsifier role of lecithin, which improved the absorption of dietary fat by improving the permeability of the intestinal membrane (Brautigan et al., 2017) and also enhanced the digestibility by the formation of small micelles (Jenkins, 1990). In contrast to our present findings, Jenkins et al. (1989) found reduced digestibility of energy, fiber, and nitrogen in lecithin-supplemented (deoiled and crude soybean lecithin @ 5.2%) groups as compared with the corn oil-supplemented group.Whereas, Jones et al. (1992) observed an increased DM and nitrogen digestibility with the addition of lecithin in the weaning pig diets. Lee et al. (2019) found decreased digestibility of DM and OM in dairy cows supplemented with LPL and whereas Huo et al. (2019) found increased digestibility of DM, OM, and CP in fattening lambs fed with 0.5% of LPL in their diet. The inconsistent results that were noticed by different researchers with respect to the digestibility of different nutrients might be due to the variations in the source and dose of the lecithin and also the duration of supplementation. Contrary to the present findings, a decreased fibre digestibility was observed by Tewari et al. (2022) with the increased level of RBCL inclusion in the diet of crossbred calves and which may be due to the higher level of RBCL used in the study, that resulted in higher crude fat and it might have negatively affected the attachment of fibre degrading bacteria over the fibre particles and thus decreased the fibre degradation. But in our present study, the digestibility of different nutrients except EE was not affected as the level of RBCL was within the normal limit and the overall fat in the diet was within the optimum range.

Nutrient balance

The balance of nitrogen, energy, Ca, and P under the different treatment groups was presented in Table 3. The N intake, excretion (Faecal and urinary),and retention (g/d) were found similar (P>0.05) among the dietary treatment groups. The results of the present experiment were in agreement with the findings of Wettstein et al. (2000b), Huo et al. (2019), and Tewari et al. (2022), where they found no effect on nitrogen balance on supplementation of different kinds of plant lecithin.Onthe contrary, an improved N retention in pigs was reported by Overland et al. (1993) on supplementation of lecithin (2%) in soy oil-based diets. Contrary to our present findings, Lee et al. (2019) observed a dose-dependent decrease in urinary N excretion in the LPL-supplemented group compared to the control. Whereas, Zhang et al. (2022) found an increased N retention and decreased faecal and urinary N excretion with the increased level of LPL in the diet of beef cattle. This might be due to the enhanced absorption of the amino acids in the small intestine due to improved permeability of the jejunum (Brautigan et al., 2017), as a result, less nitrogen was excreted in faeces and urine.

There was no significant (P>0.05) difference in Ca intake (g/d), excretion (g/d), and retention (g/d, %) among the dietary treatment groups. Whereas the intake and faecal excretion (g/d) of phosphorus (P) were significantly (P<0.01) higher in the RBCL groups than in the control. However, the urinary P excretion and retention were similar among the dietary treatment groups. Similar to the present study, Overland et al. (1994) also found no effect on Ca and P retention in pigs supplemented with a soy-lecithin (0.24%) diet.Similarly, Tewari et al. (2022) observed a significant increase in intake and faecal excretion of phosphorus in RBCL-supplemented groups as compared to the control group. It is well known that when any mineral is provided in excess, the absorption efficiency will be less and the excess amount will be excreted. The same thing was observed in the present study with respect to P intake and excretion. However, the retention of P is comparable among the different treatment groups.

The GE, DE, and ME intake (Mcal/d) were comparable among the treatment groups but numerically higher values are observed in the RBCL-supplemented groups. The faecal energy and urinary energy loss (Mcal/d) were comparable among the dietary treatment groups. The energy loss (Mcal/d) as methane was significantly ($P<0.05$) lower in the RBCL-8 followed by the RBCL-4 group as compared to the control group. Also, methane production in relation to the gross energy (GE) intake was reduced significantly in RBCL-supplemented groups (RBCL-4 & RBCL-8) relative to the control. The NE (Mcal/d) available for maintenance was comparable among the three dietary treatments. The energy balance results of the present study were in agreement with the findings of Wettstein et al. (2000b), and Huo et al. (2019). Similarly, Tewari et al. (2022) also observed a decreased methane energy loss in crossbred calves supplemented with rice bran lecithin-as compared to control animals. The decreased methane energy loss in the RBCL-supplemented group may be due to the biohydrogenation of unsaturated fatty acids in the rumen or due to the low ruminal acetate production.

Table 3. Nitrogen, energy, calcium and phosphorus balance in crossbred cattle

Attributes	Dietary groups			SEM	P value
	RBCL-0	RBCL-4	RBCL-8		
		Nitrogen balance			
N-intake and outgo (g/d)					
N intake	69.4	68.8	67.2	1.50	0.849
N faeces	33.2	35.1	35.9	1.23	0.195
N urine	16.8	15.3	14.8	0.99	0.170
N retention	19.4	18.3	16.5	1.43	0.401
N excretion (% intake)					
Faeces	47.8	51.1	53.5	1.46	0.112
Urine	23.9	22.3	21.9	1.53	0.297
N-retention (% intake)	27.9	26.6	24.6	1.02	0.378
		Energy balance			
Energy intake (Mcal/d)					
Gross energy (GE)	25.2	27.3	29.7	0.91	0.131
Digestible energy (DE)	14.5	14.9	15.8	0.44	0.511
Metabolizable energy (ME)	13.2	13.5	14.7	0.47	0.421
Faecal energy loss	10.7	12.4	13.9	0.73	0.216
Urinary energy loss	0.35	0.40	0.42	0.02	0.531
Methane energy loss	1.20[b]	0.89[ab]	0.72[a]	0.08	0.039
Methane energy (%GE)	4.78[b]	3.34[ab]	2.46[a]	0.38	0.026
Net energy (Mcal/d)	10.4	10.9	12.2	0.47	0.289

		Calcium balance			
Ca-intake and outgo (g/d)					
Ca intake	54.8	56.3	56.6	1.59	0.585
Ca faeces	30.6	33.3	34.9	1.64	0.796
Ca urine	6.87	6.04	5.53	0.42	0.086
Ca retention	17.3	17.0	16.2	0.93	0.172
Ca excretion (% intake)					
Faeces	58.0	58.2	61.6	1.73	0.587
Urine	12.9	10.8	8.13	0.74	0.128
Ca-retention (% intake)	32.6	30.9	28.6	1.89	0.364
		Phosphorus balance			
P-intake and outgo (g/d)					
P intake	30.8^{a}	40.1^{b}	49.3^{c}	2.34	<0.001
P faeces	19.0^{a}	27.1^{b}	34.2^{b}	1.54	0.002
P urine	2.30	2.80	4.59	0.52	0.173
P retention	9.50	10.15	10.49	1.07	0.344
P excretion (% intake)					
Faeces	69.2	72.2	76.7	1.65	0.417
Urine	7.45	6.86	9.44	1.09	0.633
P-retention (% intake)	30.8	23.3	21.3	1.47	0.401

[abc]Means within the row with different superscripts differ significantly (P<0.05).

Methane emission

The data on methane emission in experimental animals supplementing with graded levels of RBCL were given in Table 4. The methane production (L/d) was significantly (P<0.05) reduced in RBCL-supplemented groups than in the control group. Similar to the present findings, Wettstein et al. (2000a) reported decreased methane production in an *in-vitro* experiment when rumen liquor was treated with deoiled canola lecithin. Sontakke et al. (2014a) also reported a decrease in methane production with an increased level of rice bran lysophospholipids (ranging from 0% to 10%) in an *in-vitro* study. Recent findings from our lab also showed that decrease in methane emission in the crossbreed calves fed with RBCL (up to 6%) in their basal diet (Tewari et al., 2022). Whereas methane production (L/kg DMI, L/kg DDM, L/kg OMI, and L/kg DOM) was comparable among the treatment groups, and the level of lecithin used was quite low in the present study as compared to the above-mentioned studies, this could explain the comparable methane production in our study. However, there was a numerical decrease in methane production (L/kg DMI and L/kg W0.75) in the RBCL-supplemented groups as compared to the control, but the reduction percentage could not reach the level of significance.

In the present study, among the three groups, the RBCL-8 group has shown a 20.6% reduction in methane emission (L/d) relative to the control. Similarly, the addition of different lipid sources such as tallow or sunflower was shown to reduce methane emission by 14% whereas the diets containing sunflower seeds are shown to reduce methane by 33% in a study conducted with Angus heifers (Beauchemin et al., 2007). The reduction in methane production (L/day) and methane energy loss (Mcal/day) observed in RBCL-supplemented groups might be attributed to the direct inhibitory effect of polyunsaturated fatty acids and medium-chain fatty acids found in RBCL on fiber-degrading bacteria. This inhibition can lead to a change in the rumen fermentation pattern, resulting in reduced acetate synthesis (Lamp et al., 2018). Another possible reason is the biohydrogenation of unsaturated fatty acids, where the rumen biohydrogenation process acts as a hydrogen acceptor (electron sink), leading to a reduction in enteric methane emission (Czerkawski et al., 1966; Greening et al., 2019). In the present study, the minimal effect of RBCL on methane emission was due to the use of lower levels as compared to the earlier reported studies.

Table 4. Effect of feeding dietary TMR containing RBCL on methane emission in crossbred cattle

Attributes	Dietary groups			SEM	P value
	RBCL-0	RBCL-4	RBCL-8		
BW (kg)	308.00	319.50	311.00	11.33	0.713
Metabolic size (kg/$W^{0.75}$)	73.1	77.3	73.9	1.83	0.958
DMI (kg/d)	5.40	5.37	5.30	0.11	0.940
CO_2 produced (L/d)	1405	1413	1458	78.70	0.595
O_2 consumed (L/d)	1351	1373	1430	88.03	0.595
RQ	1.04	1.03	1.02	0.01	0.231
CH_4 production (L/d)	114^{b}	100^{ab}	90.9^{a}	4.13	0.045
CH_4 production (L/kg $W^{0.75}$)	1.59	1.32	1.26	0.09	0.275
CH_4 production (L/kg DMI)	21.3	19.1	17.3	0.98	0.271
CH_4 production (L/kg DDM)	37.8	34.6	32.1	1.69	0.433
CH_4 production (L/kg OMI)	22.9	20.6	18.7	1.05	0.286
CH_4 production (L/kg DOM)	37.5	33.8	30.9	1.71	0.319
HP (kcal/d/ kg $W^{0.75}$)	107	106	93.3	7.92	0.750
Total CH_4 energy loss (kcal/d/ kg $W^{0.75}$)	15.1	12.5	11.8	0.83	0.272
% Reduction					
CH_4 production (L/d)	-	11.9	20.6		
CH_4 production (L/kg DMI)	-	10.5	18.7		
CH_4 production (L/kg DDM)	-	8.50	14.9		

[abc]Means within the row with different superscripts differ significantly ($P<0.05$).

CONCLUSION

It can be concluded that RBCL can be used in the diet of cattle as a source of energy by replacing an equal amount of corn up to 8% in the concentrate mixture or 2.4% in the complete diet without having any adverse effect on voluntary feed intake and nutrient utilization.

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