Indian Journal of Animal Sciences **86** (1): 65–69, January 2016/Article
https://doi.org/10.56093/ijans.v86i1.55022

Effect of pre-partum supplementation of rumen protected methionine plus lysine and choline on the performance of crossbred cows

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Received: 11 November 2014; Accepted: 17 June 2015

ABSTRACT

The study was conducted to determine rumen escape potential (REP) of commercial rumen protected methionine (RPM), lysine (RPL) and choline (RPC) products and to examine the effect of supplementing RPM plus RPL and RPC on the performance of preparturient crossbred cows. Crossbred cows (27; 2nd to 4th lactation) were randomly assigned to 3 groups of 9 animals each, on the basis of most probable production ability (MPPA). Cows in control group (T1), were fed basal diet as per NRC (2001). The cows in second group (T2), were supplemented with 5 g RPM and 20 g RPL, and those in third group (T3), were fed 54 g RPC per cow/day for 40 days pre-partum. *In sacco* study revealed that REP of RPM, RPL and RPC was 75.20, 54.97 and 72.89%, respectively. Supplementation of RPM plus RPL and RPC resulted in higher body condition score (BCS) in crossbred cows on the day of parturition. Nutrient intake was similar among the groups, except that duodenal supply of methionine and lysine was higher in T2 group. Plasma triglycerides, VLDL (very low density lipoproteins) and phosphatidylcholine levels were higher in T2 and T3 groups while vitamin E level was higher in T3 group, on the day of parturition, indicating better immune status. It was concluded that fortification of ration with RPM plus RPL and RPC resulted in improved body condition score, duodenal supply of methionine and lysine and better immune status in cows during prepartum period.

Key words: Choline, Cows, Lysine, Rumen protected methionine

Methionine and lysine are considered to be the first 2 limiting amino acids for milk production (NRC 2001). In India, most of the feed ingredients rich in ruminally undegradable protein are from vegetable sources and are deficient in one or more essential amino acids. Feedstuffs with low rumen degradability and/or high quality protein with a well balanced amino acid profile (such as meat meal, bone meal, fish meal etc.) are expensive and legislatively banned for use in cattle feed in India. The only practical way left, to reach the required level and ratio of essential amino acids, is dietary supplementation of rumen protected amino acids.

Choline, an important rumen protected nutrient, is involved in the transport of fat from liver and is required for the synthesis of phosphatidylcholine, which is a phosholipid found in the membranes of very low density lipoproteins. The requirement of choline for dairy cows is still unknown (NRC 2001); 28% of the absorbed methionine is used for choline synthesis. Potential exists to improve amino acid nutrition of the ruminants by changing the choline status. The choline is degraded extensively by ruminal microbes hence, it is to be protected from ruminal degradation. Under Indian feeding conditions, scanty information is available on RPM and RPL as well as RPC supplementation in pregnant cows. The present experiment was therefore designed to determine the rumen escape potential (REP) of RPM, RPL and RPC and to study the effect of supplementing these on nutrient intake, body weight changes and haemato-biochemical parameters in crossbred cows during prepartum period.

MATERIALS AND METHODS

Experimental animals, feeds and management: 27 crossbred cows (*Bos taurus* × *Bos indicus*) were divided into 3 groups having 9 cows in each group, on the basis of MPPA and lactation number (2nd to 4th). Animals in control group (T1; MPPA, 4,119 kg) were fed with chopped wheat straw (particle size: 1.5 to 2.0 cm), chaffed maize fodder (particle size: 2.0 to 2.5 cm) and compound concentrate mixture as per requirements (NRC 2001). Animals in second group (T2; MPPA, 4,120 kg) were fed with the same basal ration with the additional supplementation of 5 g RPM (net 1.98 g intestinally deliverable) plus 20 g RPL (net 4.42 g intestinally deliverable)/cow a day. Animals in third group (T3; MPPA, 4,108 kg) were fed with the same ration as

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group T1, with the additional supplementation of 54 g RPC (net 10 g intestinally deliverable)/cow a day. The concentrate mixture was offered twice a day in equal parts at the milking time i.e. 05:00 and 18:00 h. RPM, RPL and RPL were fed through concentrate mixture at one time i.e., 05:00 h. Fresh maize fodder was fed at 10:00 and 19:00 h in addition to wheat straw. Leftover, if any, was weighed next morning. The dietary treatments were continued for 40 days before parturition, including an adaptation period of 10 days. Concentrate mixture contained maize 33%, groundnut cake 21%, mustard cake 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%. Animals, twice in a week, were left free for 1 h in open paddock for exercise. All the management practices were approved by the Institutional Animal Ethics Committee guidelines.

Rumen escape potential (REP) of commercial RPM, RPL and RPC products: RPM, RPL and RPC were obtained commercially. The products were in the form of encapsulation with fatty acids and prepared by spray freeze drying technology. Rumen protection level of the RPM, RPL and RPC was estimated using *in sacco* nylon-bag technique (Mehrez and Orskov 1977, Osuji *et al*. 1993, Noziere and Michalet-Doreau 2000). Effective degradability (ED) and REP were calculated.

Nutrient intake and body weight record: Animals were kept in well ventilated, roofed and concrete floor shed having individual pens for each animal. All the cows were offered weighed quantity of concentrate mixture, wheat straw and maize fodder. Amount of feed offered and refusals from all the cows were weighed daily and sampled at weekly intervals for subsequent estimation of DM. The animals were weighed fortnightly for 2 consecutive days before offering feed and water to estimate body weight changes. BCS was recorded based on the score chart given by NRC (2001).

Chemical analysis: The dried samples of concentrate mixture, wheat straw, maize fodder and residue were ground and analysed for the proximate composition (AOAC 2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as per Van Soest *et al*. (1991) with heat stable α-amylase used only for concentrate mixture. Amino acid content of experimental feedstuffs was determined using high performance liquid chromatography (HPLC) fitted with absorbance detector (254 nm).

Blood collection and analysis: About 10 ml blood was collected from each cow by jugular venipuncture in the morning before offering feed and water at fortnightly intervals (-30^{th} to 0^{th}day) into heparinized vaccutainer tubes. The plasma was separated by centrifugation of the blood at 2,400 rpm for 15 min, stored in plasma vials at – 20° C and subjected to the analyses of glucose, blood urea nitrogen (BUN), cholesterol, triglycerides and VLDL (very low density lipoproteins), spectrophotometrically using commercial kits. Non-esterified fatty acids (NEFA) concentration was estimated as per Shipe *et al.* (1980). Vitamin E was estimated using HPLC and phosphatidycholine was determined spectrophotometerically (Sharma and Erdman 1989).

Statistical analysis: The data generated during the experiment were subjected to the one-way analysis of variance (ANOVA) using the SAS (2003) software employing the linear model. The post-hoc comparison of means were done for the significant difference ($P<0.05$) by Tukey's *b*.

RESULTS AND DISCUSSION

Rumen escape potential of RPM, RPL and RPC: The degradation potential of RPM, RPL and RPC was determined by *in sacco* nylon bag technique to find out the stability of commercial products in the rumen (Table 1). The total methionine in commercial RPM product was 52.68%. The REP of RPM was 75.20% with ED of 24.80%. The total lysine content in the commercial RPL product was 40.20% with ether extract content being 49.00%. The products were in the form of encapsulation with fatty acids and prepared by spray freeze drying technology. The REP of RPL was 54.97% with ED being 45.03%. Commercial RPC product contained 25.44% choline. The REP of the product was 72.89% with ED of 27.11%.

Chemical composition and amino acid profile of experimental feeds: The chemical composition of feed ingredients (Table 2) was within the normal range for Indian feedstuffs (ICAR 2013).

Table 1. Characteristics of commercial RPM, RPL and RPC products

Particulars (% DM)	RPM	RPL	RPC
Methionine	52.68±0.04	0	0
Lysine	0	40.20±0.46	0
Choline	0	0	25.44±0.27
Fat	44.90±0.32	49.00±0.50	73.95±0.40
Degradation (%)			
0 h	0.77±0.28	15.34±0.88	16.80±0.49
3 h	20.25±0.91	44.64±0.52	24.84±0.21
6 h	22.01±0.60	46.41±0.16	26.91±0.32
12 h	28.37±0.68	48.04±0.25	29.43±0.08
24 h	44.17±2.28	48.99±0.16	30.83±0.13
ED	24.80±0.57	45.03±0.22	27.11±0.03
REP	75.20	54.97	72.89

ED,Effective degradability; REP, rumen escape potential.

Table 2. Chemical composition of experimental feedstuffs, % DM basis

Item	Concentrate	Maize fodder	Wheat straw
OM	91.26	95.64	92.34
CP	21.00	10.52	3.50
EE	3.75	1.67	0.33
Ash	8.74	4.36	7.66
NDF	35.96	48.64	73.45
TDN%*	69.2	65.3	46.6

*Calculated as per NRC (2001).

Table 3. Amino acid profile of experimental diet,% CP basis

Feed	Essential amino acids											Non essential amino acids						
	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TRP	VAL	ALA	ASP	GLU	GLY	PRO	SER	TYR
Concentrate	5.82	0.89	1.64	2.92	5.71	3.43	1.50	3.68	2.74	1.07	4.23	3.68	7.36	11.50	3.78	6.10	3.65	3.56
Maize fodder	4.43	0.71	1.24	3.83	6.62	3.60	1.45	4.26	3.29	0.52	5.04	5.15	7.94	8.79	4.09	5.19	3.46	3.72
Wheat straw	5.33	1.18	1.66	3.11	5.10	3.11	1.23	4.03	2.64	1.57	4.48	3.42	5.37	14.97	3.80	6.76	3.27	3.35

The amino acid profile of feeds used in the experiment (Table 3) revealed that concentrate mixture, maize fodder and wheat straw contained 1.50, 1.45 and 1.23% methionine, whereas lysine content was 3.43, 3.60 and 3.11% (CP basis), respectively. All the values were within the normal range as reported by NRC (2001) and NDDB (2012).

Body weight changes and BCS: Fortnightly changes in body weight of prepartum crossbred cows revealed that in the first fortnight (–30th to -15th day prepartum), the animals gained 1.5, 1.4 and 1.7% body weight in groups T1, T2 and T3, respectively (Table 4). However, in the second fortnight (–15th to 0th day), the loss in body weight was 11.4, 12.4 and 12.9% in T1, T2 and T3 groups, respectively, due to parturition and release of the foetal membranes. There was no significant effect of RPM, RPL and RPC supplementation on body weight changes among the 3 groups. The results are in concordance with Lara *et al*. (2006) who reported no effect of supplemental RPM on body weight and BCS in Holstein cows. Similar findings were observed by Sheikh (2012) on supplementation of RPC in lactating crossbred cows.

BCS was numerically higher in T2 and T3 groups as compared to T1 group, on the day of parturition (Table 4). BCS is actually a logistic tool for assessment of nutritional status of animal and their management for optimal performance. The maintenance of an optimal BCS *vis-a-vis* lactation stage, milk yield, and nutrition and health status is the most important aspect of dairy cow management that facilitates a healthy transition from pregnancy to lactation. Condition loss indicates intensive mobilization of body tissue during the first few weeks of lactation. BCS at calving is also a reliable indicator of reproductive performance. Cows that are having too high BCS at calving or those losing excess body weight are more likely to have a prolonged interval to first oestrus; thereby prolonging days open (Roche 2006).

Table 4. Fortnightly body weight changes (%) and body condition score of crossbred cows during prepartum period

Day	T1	T2	T3
Body weight (kg)			
–30	509.8±22.9	498.6±10.1	470.8±26.5
–15	517.4±23.0	505.3±9.6	478.8±26.0
0*	459.2±20.3	442.7±11.5	417.0±23.8
% Change in body weight			
–15	+ 1.5±0.1	+ 1.4±0.2	+1.7±0.3
0*	–11.4±0.7	–12.4±0.7	–12.9±1.0
BCS			
0*	3.47±0.11	3.69±0.06	4.14±0.04

Values are given as mean±SE; *, Day of parturition.

Nutrient intake: The DMI during first (–30 to –15 days prepartum) and second (–15 to 0 days prepartum) fortnight showed no significant difference among the groups (Table 5), indicating no effect of RPML and RPC supplementation on DMI of prepartum cows. Socha *et al*. (2005) and Sheikh (2012) also reported no effect of RPM and RPC supplementation, respectively, on the DMI of cows. Contrary to this, several workers (Christensen *et al*. 1994, Piepenbrink *et al*. 1996) reported that DMI was depressed by feeding RPM, but, this depression was reversed when RPL was also supplemented. Reduction in DMI was attributed to lower microbial activity, and, thereby, reduced digestion in the rumen. However, in the present study, analysis of the RPM, RPL and RPC products did not reveal any contaminants that could affect microbial growth. So

Table 5. Prepartum average nutrient intake of crossbred cows

Parameter	Fortnight	T1	T2	T3
DM intake (kg/d)	1	9.33±0.50	8.87±0.28	9.28±0.38
	2	8.21±0.54	7.75±0.24	7.66±0.25
CP intake (kg/d)	1	1.17±0.04	1.14±0.04	1.23±0.07
	2	1.09±0.05	1.04±0.03	1.11±0.06
RUP intake (kg/d)	1	0.40±0.02	0.39±0.01	0.42±0.02
	2	0.36±0.02	0.34±0.02	0.37±0.02
RDP intake (kg/d)	1	0.77±0.03	0.75±0.02	0.81±0.05
	2	0.72±0.03	0.70±0.03	0.74±0.04
MP intake (kg/d)	1	0.76±0.03	0.74±0.02	0.80±0.05
	2	0.70±0.03	0.67±0.03	0.72±0.04
Duodenal methionine flow (% of MP)	1	1.90 ^{a}±0.03	2.22 ^{b}±0.02	1.94^{a}±0.01
	2	1.97 ^{a}±0.02	2.30 ^{b}±0.02	1.97$^{a\pm}$0.01
Duodenal lysine flow (% of MP)	1	6.44^{a}±0.03	7.09^{b}±0.04	6.45^{a}±0.03
	2	6.57 ^{a}±0.06	7.35^{b}±0.07	6.57^{a}±0.02
NE_L intake (Mcal/d)	1	13.59±0.55	13.33±0.39	14.24±0.64
	2	12.36±0.61	11.60±0.50	12.58±0.56
ME intake (Mcal/d)	1	21.78±0.90	21.28±0.63	22.73±1.00
	2	19.78±1.00	18.50±0.80	20.07±0.88

Means with different superscripts in a row differ significantly ($P<0.05$).

Table 6. Plasma essential amino acid profile (µmol/dl) of crossbred cows on the day of parturition

Amino acid	T1	T2	T3
Arginine	7.63±0.56	8.29±0.21	7.89±0.34
Cysteine	1.61±0.45	2.11±0.29	1.59±0.26
Histidine	4.66±0.49	4.31±0.43	4.83±0.14
Isoleucine	10.79±0.83	9.54±0.72	9.20±0.53
Leucine	15.11±1.08	16.18±0.61	14.64±0.53
Lysine	13.28±0.86	14.25±0.84	13.36±0.52
Methionine	5.39±0.32	5.93±0.27	5.88±0.37
Phenylalanine	8.26±0.62	9.40±0.87	7.66±0.17
Threonine	11.84±0.61	12.50±0.85	11.82±1.01
Valine	15.21±0.91	16.20±0.53	15.34±0.44

Table 7. Plasma metabolites of crossbred cows during prepartum period

Parameter	Day	T1	T2	T3
Glucose, mg/dl	–30	57.19±1.20	56.06±0.86	58.39±0.20
	–15	56.55±0.85	54.14±0.82	55.42±0.32
	0*	46.02±1.36	45.86±0.53	49.08±0.75
NEFA, mg/L	–30	69.53±1.14	67.63±1.05	70.89±0.81
	–15	91.89^{c}±1.32	77.58^{a}±2.89	83.44 ^{b}±2.42
	0*	115.94±1.74	112.14±2.55	107.42±3.30
Triglycerides, mg/dl	–30	17.30±0.84	18.66±0.31	18.31±0.37
	–15	16.27^{a}±0.58	18.24^{b}±0.23	16.56 ^{a}±0.33
	0*	16.12^{a}±0.67	17.89^{b}±0.43	17.19^{b}±0.26
VLDL, mg/dl	–30	3.46±0.17	3.73±0.05	3.66±0.07
	–15	3.25^{a}±0.12	3.65^{b}±0.05	3.31^{a}±0.07
	0*	3.22^{a}±0.13	3.58^{b}±0.09	3.44^{b}±0.05
Phosphatidyl-choline, µg/ml	–30	152.10±5.49	149.39±5.80	148.43±4.43
	–15	122.35^{a}±2.54	133.26^{b}±7.26	130.61^{b}±2.28
	0*	95.58^{a}±2.31	109.00^{b}±5.57	118.43^{c}±1.48
Vitamin E, µg/ml	–30	1.07±0.11	1.03±0.03	0.87±0.16
	–15	0.81±0.07	0.79±0.04	0.81±0.02
	0*	0.68^{a}±0.03	0.66^{a}±0.03	1.13 ^{b}±0.08
Cholesterol, mg/dl	–30	166.80±6.13	167.37±5.66	169.33±5.24
	–15	167.25±5.90	172.74±4.57	164.47±7.01
	0*	171.33±5.53	173.88±5.97	166.32±6.57
BUN, mg/L	–30	14.08±0.20	13.38±0.42	13.88±0.23
	–15	19.39±0.87	19.03±0.55	18.48±0.99
	0*	20.34±0.90	20.75±0.34	21.84±0.59

Means with different superscripts in a row differ significantly (P<0.05); 0*, day of parturition.

the reason for less DMI may be advanced stage of pregnancy as the foetus occupies most of the space in abdominal cavity. CP intake also followed the trend similar to DMI. There was no significant difference in RUP, RDP, MP, NE_L, MP and ME intake among the 3 groups. However, the methionine and lysine supply at duodenum was higher (P<0.05) in T2 group than T1 and T3 groups. In transition period, feed intake decreases while protein and energy requirements increase due to rapid growth of the conceptus, leading to an increased demand of methionine and lysine. Consequently, in order to prevent a change in N balance, the duodenal flow of methionine and lysine should be stepped up.

Plasma amino acid profile: The levels of essential amino acids on the day of parturition were similar among the 3 groups (Table 6). However, plasma lysine and methionine concentrations tended to increase in T2 and T3 groups supplemented with RPM plus RPL and RPC, respectively. Similar findings were reported by Berthiaume *et al.* (2001) and Swanepoel *et al.* (2011) on supplementation of diet with RPM and RPL, respectively, in dairy cows.

Blood metabolites: The plasma glucose levels followed a decreasing trend as the parturition approached in all the 3 groups (Table 7). However, no significant difference was observed in glucose levels among the groups. The plasma glucose levels remained within the normal physiological range at every stage of the experiment. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body that does not allow appreciable change in glucose level.

The plasma NEFA levels were similar among the 3 groups at 30 days prepartum. However, plasma NEFA levels were lowest (P<0.05) in T2 group at 15 days prepartum followed by T3 group and were highest (P<0.05) for T1 group. The decrease in plasma NEFA concentration reflected the fact that there was esterification of NEFA to triglycerides, which are subsequently exported as VLDL for which phosphatidylcholine is required. However, NEFA levels in all the groups showed an increasing trend as the pregnancy advanced. Higher plasma NEFA concentration observed in multiparous cows towards parturition suggested that cows were mobilizing more stored energy to support increasing demand of growing conceptus.

Plasma triglyceride and VLDL levels were higher (P<0.05) in T2 group than T1 and T3 groups at 15 days prepartum. However, on the day of parturition, the level of triglycerides and VLDL was higher (P<0.05) in both T2 and T3 groups than T1 group. The higher VLDL content in RPC supplemented group might be due to antilipolytic nature of choline as indicated by high triglyceride concentration in this group. Also RPC supplementation resulted in an increased transport of lipids from the liver as fat is transported out of the liver by VLDL whose levels are also elevated in T3 group. Amrutkar (2011) has also shown that RPM supplementation facilitates lipoprotein synthesis in liver, which may be responsible for higher plasma VLDL concentration in cows.

There was increase (P<0.05) in plasma phosphatidylcholine (a key component in the synthesis of VLDL) level in T2 and T3 groups 15 days prior to parturition. The increase in plasma phosphatidylcholine in T2 group may be due to utilization of the absorbed methionine for *de novo* synthesis of choline (Emmanuel and Kennelly 1984). However, on the day of parturition, the plasma phosphatidylcholine levels were highest (P<0.05) in T3 group (supplemented with RPC) followed by T2 and were lowest (P<0.05) in T1 group, indicating that the RPC was being absorbed postruminally and was available to cows at tissue level.

The plasma vitamin E and cholesterol levels were within the normal physiological range. The vitamin E levels were higher (P<0.05) in T3 group than T1 and T3 groups, on the day of calving, indicating positive effect of RPC supplementation on the immune system. Chatterjee and Walli (2003) and Sheikh (2012) also reported that RPC supplementation in cows leads to improved immunity. The cholesterol levels were numerically higher in T2 group than that in T1 and T3 groups. Higher cholesterol concentration is associated with better reproductive performance in high yielding dairy cows, as cholesterol acts as a precursor of steroid hormones. RPC supplementation, however, had no significant effect on cholesterol level.

The BUN levels did not differ in response to dietary treatment, suggesting that overall N utilization was similar in all the groups. In line with our data, nonsignificant differences in BUN levels on supplementing RPM plus RPL were reported by Socha *et al*. (2005) in prepartum cows. As far as RPC supplementation is concerned, earlier report by Mohsen *et al.* (2011) has indicated nonsignificant decline in BUN level on RPC supplementation in cows. However, in the present study, there was an increasing trend in plasma BUN concentration as the cows progressed towards parturition. This may be due to the fact that BUN is an indicator of efficient protein balance and is typically increased in cows deficient in energy.

It could be concluded that RPM plus RPL and RPC supplementation in prepartum dairy cows improved the BCS on the day of parturition. The triglyceride, VLDL and phosphatidylcholine levels were higher (P<0.05) in groups supplemented with RPM plus RPL and RPC on the day of calving. The higher plasma vitamin E level in RPC supplemented group indicated better immune status of the animals in this group. Thus, supplementation of diet with RPM plus RPL and RPC can be used as a tool to improve the BCS and health of cows during the prepartum period.

ACKNOWLEDGMENT

The authors are grateful to the Director, NDRI for providing necessary facilities to carry out this research work.

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