Agric Res (December 2015) 4(4):396–404
DOI 10.1007/s40003-015-0178-2

FULL-LENGTH RESEARCH ARTICLE

Dietary Supplementation of Rumen-Protected Methionine, Lysine and Choline Improves Lactation Performance and Blood Metabolic Profile of Karan-Fries Cows

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Received: 29 January 2015 / Accepted: 31 August 2015 / Published online: 18 November 2015

Abstract Twenty seven crossbred (Karan-Fries) cows between second and fourth lactation with most probable production ability of around 4500 kg milk were divided into three similar groups. Animals in the Control group were offered with a basal diet consisting of threshed wheat straw, chopped green maize forage and compounded concentrate mixture as per their requirements. In addition to basal diet, animals in second group (rpMetLys) were fed with 5 g of rumen-protected methionine and 20 g of rumen-protected lysine, and in third group (rpCholine), 54 g of rumen-protected choline was supplemented 40 days before and 120 days after calving. Results revealed higher ($P < 0.01$) intake of various nutrients along with better body condition score in rpMetLys group. Furthermore, milk yield and milk component (fat, protein and lactose) yield were higher ($P < 0.01$) in both the treatment groups with higher ($P < 0.01$) milk choline concentration in rpCholine group. Monounsaturated fatty acids and unsaturated fatty acids in milk tended to be higher ($P < 0.05$) in rpCholine group. Plasma metabolites like glucose, non-esterified fatty acids, cholesterol and plasma urea nitrogen were similar among all groups. Concentration of triglycerides and very low density lipoproteins was lower ($P < 0.01$) in rpMetLys and rpCholine than Control group. However, phosphatidylcholine and vitamin E levels were higher ($P < 0.01$) in rpCholine than other two groups. Methionine levels were higher ($P < 0.01$) in rpMetLys and rpCholine groups, whereas lysine was increased ($P < 0.01$) only in the former. Moreover, days open (service period) was decreased ($P < 0.01$) in animals belonging to rpCholine group. It was concluded from the present study that supplementation of rpMetLys and rpCholine to high-yielding crossbred cows was beneficial in terms of improving milk yield, composition as well as metabolic health status during early lactation period under Indian situation.

Keywords Choline · Crossbred cows · Lysine · Methionine · Milk yield

Abbreviations
AA: Amino acids; ADF: Acid detergent fibre; BCS: Body condition score; DMI: Dry matter intake; ED: Effective degradability; EE: Ether extract; FCM: Fat-corrected milk; FID: Flame-ionisation detector; MCP: Microbial crude protein; ME: Metabolisable energy; MP: Metabolisable protein; MUFA: Monounsaturated fatty acids; MUN: Milk urea nitrogen; NDF: Neutral detergent fibre; NEFA: Non-esterified fatty acids; PUFA: Polyunsaturated fatty acids; PUN: Plasma urea nitrogen; RDP: Rumen degradable protein; rpCholine: Rumen-protected choline; rpLys: Rumen-protected lysine; rpMet: Rumen-protected methionine; SFA: Saturated fatty acids; UDP: Undegradable dietary protein; UFA: Unsaturated fatty acids; VLDL: Very low density lipoproteins

Introduction

Current system of protein rationing in ruminants aims at balancing for amino acid requirements rather than solely relying upon total crude protein (CP) content [21, 26, 28]. Specifically, protein requirements for lactating cows are

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fulfilled by the sum of digestible microbial protein (MCP) synthesised in the rumen as well as digestible undegradable dietary protein (UDP), together constituting metabolisable protein (MP), which is actually the truly available amino acids for milk production [21]. Though a great deal of research has been done in the past to improve MCP in the rumen, protein requirement during lactation far exceeds daily synthesis of MCP [32]. Therefore, UDP fraction in the diet is generally increased during early lactation to meet such a high demand for milk synthesis [31, 32]. However, a majority of UDP sources used in the tropics are of vegetable origin, which is often deficient in critical amino acids (AA) like methionine [29]. Nonetheless, the nutritive value of MP is determined by its profile of essential AA mainly lysine (Lys) and methionine (Met) which are needed in 3:1 proportion [21]. Research in the past has shown that by improving the profile of AA in the MP through dietary supplementation of rumen-protected AA mainly Lys and Met, it is possible to minimise CP level of the ration to 16 % [6], while maintaining milk yield and milk protein yield during early lactation.

Choline functions as a lipotropic factor, plays a role in carnitine synthesis and is a biological methyl donor required for many cellular biochemical reactions [24]. Specifically, rpCholine decreases hepatic intracellular triacylglycerol stores, increases liver glycogen synthesis and lipoprotein secretion as well as optimises methyl group metabolism [24]. Although NRC [21] has not specified any dose level for choline, it has been identified as a critical nutrient during transition period [16].

A vast majority of research has focused on high-yielding cows of temperate world, but, information on the responses to rumen-protected methionine—(rpMet), lysine (rpLys) and choline (rpCholine) in cows kept under tropical conditions is scarce.

Crossbred cattle-like Karan-Fries are known for their good milk producing ability [14]. However, efforts have not been devoted much to understand nutritional needs of such crossbreds to completely exploit their genetic potential. Moreover, quantitative requirements of rumen-protected amino acids and choline to improve performance parameters of early lactating cows have not been understood completely [19, 24]. As nutrient requirements differ widely between temperate and tropical cows [23], the current study was undertaken with the hypothesis that supplemental rpMet, rpLys and rpCholine improve milk yield, milk component yield and metabolic status of Karan-Fries cows. The main objectives were to evaluate the effect of supplementation of rpMet, rpLys and rpCholine on milk yield, composition, fatty acid profile, efficiency of nutrient utilisation, plasma amino acid status and blood measures in early lactating Karan-Fries cows.

Materials and Methods

Location of Study Area

This study was conducted at the experimental animal shed of ICAR-National Dairy Research Institute, Karnal, India located at 29° 42′ 20 s N and 76° 58′ 52.5 s E at an altitude of 227 m amsl. Minimum and maximum ambient temperature range from near freezing point in winter to 45 °C in summer with an annual rainfall of 700 mm. The present experiment was conducted in summer as well as winter with daily minimum and maximum temperature averaging 5.6 and 40 °C.

Experimental Animals, Feeding and Management

Twenty-seven apparently healthy, previously dewormed Karan-Fries (*Bos taurus* × *Bos indicus*) cows in their early lactation (second to fourth parity) were selected from institute's Livestock Research Centre and divided into three similar groups based on most probable producing ability of around 4500 kg. The animals were individually identified by numbered ear tags, tethered with nylon rope individually in a well-ventilated stall provided with uniform management practices and having facilities for individual feeding. Antiseptic solution (phenyl) was sprayed at regular intervals on the floor to ensure maximum hygiene. Animals in Control group was fed with threshed wheat straw (particle size: 1.5–2.0 cm), chopped green maize (African Tall variety; particle size: 2.0–2.5 cm) and compounded concentrate mixture to fulfil their nutrient requirements [21] throughout 120 days of experiment. A daily dose of 5 g rpMet (MetiPEARL^{TM}, net 1.98 g intestinally deliverable) and 20 g rpLys (LysiPEARL^{TM}, net 4.42 g intestinally deliverable) were supplemented orally to animals of second group (rpMetLys) and 54 g of rpCholine (CholiPEARL^{TM}, net 10 g intestinally deliverable) to third group, by mixing them with concentrate mixture at 11:30 h ensuring the complete intake of supplements. Supplementation was started 40 days prior to calving and continued until 120 days post-partum to document performance outcomes of cows. All supplemental products used in the study were procured from Kemin Industries Inc. (Chennai, India).

Green maize forage was offered separately, while wheat straw was mixed with concentrate mixture and fed as per weekly calculated requirements. Concentrate mixture was offered thrice daily during milking time, i.e. 05:00, 11:30 and 18:00 h. Fresh green maize forage was fed at morning (10:00 h) and evening (19:00 h) in addition to wheat straw, which was offered at 07:30 h. All the feedstuffs were weighed daily, using spring

balance before feeding. Residues of feeds, if any, were weighed next morning and oven dried (100 °C, 24 h) to calculate daily dry matter intake (DMI). Clean and fresh drinking water was made available ad libitum thrice daily after milking. The Institutional Animal Ethics Committee approved all animal-related management practices carried out in this study.

Characteristics of Supplements

Rumen escape potential of the rpMet and rpLys was determined using an in sacco nylon-bag technique [17, 21], expressed as degradability on hourly intervals and finally the effective degradability (ED).

$$P = a + b\,(1 - e^{-ct}),$$

where P is the degradability, t is the time, a is the intercept on Y axis, b is the potentially degradable fraction and, c is the degradation rate or rate constant.

Rumen degradable protein (RDP) and UDP fractions of supplements as well as feedstuffs were computed as per the equations proposed by NRC [21].

Observations Recorded

Daily DMI of individual cows was calculated as the difference between DM offered and DM in the residue. Intake of various nutrients like crude protein (CP), MP, RDP, UDP and metabolisable energy (ME) was calculated in accordance with NRC [21]. Total daily milk yield was recorded for each cow separately by pooling milk yield of three times. Four percent of fat-corrected milk (FCM) was calculated based on actual fat percent and total milk yield. Animals were weighed for two consecutive days in the morning before access to feed and water, and their average was taken as body weight for the particular fortnight. Fortnightly, body condition score (BCS) of each animal was determined considering vertebral column, spinous process, tail-head region and ribs, and presented on five-point scale [21]. Reproductive performance was compared among the groups by taking different variables like commencement of cyclicity (days), days open, artificial insemination (AI) per conception and conception rate as detailed by Tyagi et al. [33]. Cows were observed for 30-minute period, by the expert technician, thrice daily (0700, 1600 and 2100 h) for the signs of estrus, and inseminated accordingly after 60-day post-partum. Approximately 90 days after AI, pregnancy diagnosis was carried out by rectal palpation of the uterus. Conception rate was defined as the number of cows pregnant per 100 inseminations, and pregnancy rate as the number of cows pregnant per 100 cows in the group.

Sampling and Biochemical Analysis of Blood

Blood samples from each animal (about 10 mL) prior to morning feeding and watering were collected every fortnightly in 16 × 100 mm heparinised vacutainers (Becton–Dickinson, Rutherford, NJ) via jugular venipuncture, mixed well and brought to the laboratory by placing in ice box. Samples were centrifuged (Heraeus, Germany) at $3000\times g$ for 15 min at 4 °C to recover plasma and stored at −20 °C until analysed.

Sampling and Composition of Milk

One hundredth portion of total milk per cow (evening, morning and noon) was sampled every fortnightly to analyse major milk components (fat, protein and lactose) using precalibrated Milk Analyser (LactoStar, FUNKE GERBER, Article No 3510, Berlin, Germany).

Milk Urea Nitrogen (MUN)

MUN was determined by a modified colorimetric *p*-Dimethylaminobenzaldehyde (DMAB) assay [2]. In this method, urea forms a yellow complex with the DMAB reagent in an alcohol low acidic solution at room temperature, and the intensity of colour was measured spectrophotometrically at 425 nm.

Fatty Acid Analysis

From the pooled milk samples, fatty acid analysis was done [18] based on the method of O'Fallon et al. [22]. Fatty acids were quantified by gas liquid chromatography fitted with flame ionisation detector (FID) and a capillary column (50 m long). Initial temperature of the column was 140 °C. Helium was used as a carrier gas.

Laboratory Analysis

Chemical Composition and Fibre Fractionation of Feeds and Residues

Representative subsamples of the feeds offered and left-over residuals were oven dried (65 °C, 48 h), ground to pass through 1 mm screen and analysed for DM (ID 934.01), CP (Kjeldahl N × 6.25, ID 984.13), ether extract (EE, ID 920.39) and ash (ID 942.05) following standard methods of AOAC [1]. Fibre fractions like neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the procedure of Van Soest et al. [34] with heat-stable α-amylase (Sigma A3306, Sigma-Aldrich, USA) used only for concentrate mixtures, and expressed

inclusive of residual ash. Hemicellulose was the difference between NDF and ADF. Total carbohydrates and non-fibrous carbohydrates were calculated by difference [30].

Blood Biochemical Analysis

Plasma from collected blood samples was subjected to the analysis of blood glucose, plasma urea nitrogen (PUN), cholesterol, triglycerides and very low density lipoproteins (VLDL) spectrophotometrically using commercial kits obtained from Span Diagnostic Limited (Surat, India). Procedure for colorimetric estimation of non-esterified fatty acids (NEFA) was standardised using the extraction method of Itaya and Ui [12]. Vitamin E was estimated using isocratic HPLC (Waters India Pvt. Ltd.) method [8]. Plasma amino acids were estimated by HPLC [1] equipped with Picotag® assembly. Individual amino acids were identified by comparing the retention times with that of standard (Sigma-M 8710, Sigma-Aldrich, USA). For plasma and milk choline assay, colorimetric method was used employing choline bitartrate as standard (Sigma C-2654, Sigma-Aldrich, USA) and expressed as phosphatidylcholine.

Statistical Analysis

Replicated data obtained in the experiment were tabulated as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SigmaStat for Windows version 3.10 (Systat software Inc., USA). The means were compared at 5 and 1 % level of probability to declare statistical significance at $P < 0.05$ and $P < 0.01$, respectively.

Results and Discussion

In this study, we investigated the response of crossbred cows to supplemental critical nutrients like rumen-protected methionine plus lysine and choline during early lactation. To the best of our knowledge, this is the first report to document such a response among tropical crossbred (Karan-Fries) cows.

Chemical Composition of Feeds and Characteristics of Dietary Supplements

Chemical composition and cell wall fractions of concentrate mixture, green maize and wheat straw are furnished in Table 1. Average nutrient content for all feeds was within normal range as reported in table values for Indian feeds [11]. Furthermore, characteristics of dietary supplements, i.e. rpMet, rpLys and rpCholine, revealed sufficiently high rumen escape potential of 752, 550 and 729 g/kg, respectively (Table 2).

Table 1 Average chemical composition (g/kg DM), energy and protein values of experimental feeds ($n = 5$ per feed)

Particular	Concentrate mixture[a]	Green maize forage	Wheat straw
Organic matter	912.6	956.4	923.4
Crude protein	210.0	105.2	35.0
Ether extract	37.5	16.7	3.3
Ash	87.4	43.6	76.6
Neutral detergent fibre	359.6	486.4	734.5
Acid detergent fibre	112.3	252.6	506.5
Hemicellulose	247.3	233.8	228.0
Total carbohydrates	665.1	834.5	885.1
Non-fibrous carbohydrates	305.5	348.1	150.6
Metabolisable energy (MJ/kg)	10.39	9.79	6.99
Rumen degradable protein (g/kg CP)	694.1	606.1	318.8
Undegradable dietary protein (g/kg CP)	305.9	393.9	681.2

[a] Composition (g/kg, as mixed): Maize 330, groundnut cake 210, mustard cake 120, wheat bran 200, de-oiled rice bran 110, mineral mixture 20 and salt 10

Body Weight Changes, BCS and Nutrient Intake

Three groups of experimental animals differed ($P < 0.05$) in body weight changes (Table 3). Live weight was decreased in Control animals, while animals in both the supplemental groups had gained body weight, which was the highest for rpCholine group. Fortnightly BCS showed no difference between groups Control and rpCholine, whereas it was higher ($P < 0.01$) for group rpMetLys (Table 3). Loss of body weight during early lactation is attributed to the massive mobilisation of depot fats in order to offset negative energy balance. BCS is a logistic tool for assessing the nutritional status of dairy cows and their management for optimal performance. In the present study, BCS reflected the changes in the body weight, both in supplemented and Control groups.

DMI differed ($P < 0.01$) between Control and rpMetLys groups, but was similar with that of rpCholine group. Similarly intakes of CP, UDP, MP followed the same trend, which were the highest in rpMetLys group. Intakes of RDP ($P < 0.01$) and ME ($P < 0.05$) were higher in rpMetLys but similar between that of Control and rpCholine (Table 3). Results are consistent with those of Nichols et al. [20], who reported a higher DMI (30.0 vs. 27.7 kg/day; $P < 0.01$) in corn-distillers grain diet than soybean meal diets upon supplementation of rpMetLys in multiparous Holstein cows. In contrast, Benefield et al. [4]

documented a declining trend (28.4 vs. 28.0) in DMI when multiparous Holstein cows were supplemented with 12 g of rpMet, which they ascribed to the slight sulphur odour of commercial rpMet source (Mepron®) used in their experiment. More recently, Zanton et al. [37] concluded from a meta-analysis study that DMI differs due to the source of rpMetLys in dairy cows. However, no such differences were observed in our study to cause a depression in DMI. Furthermore, rpCholine supplementation had no effect on DMI, as has been previously reported [9, 36]. Higher intake of other nutrients (CP, RDP, UDP, MP and ME) observed in rpMetLys group is a direct consequence of higher DMI.

Milk Production, Composition and Nutrient Efficiency

Data on milk production indicated that animals in rpMetLys and rpCholine had higher ($P < 0.01$) yield of milk as well as 4 % FCM, and were the highest in latter (Table 4). Similar to milk yield, milk component (fat, protein and lactose) yields were also higher in two supplemental groups than that of Control. Perusal of data on fatty acid profile showed lower ($P < 0.01$) total saturated fatty acids, higher ($P < 0.01$) mono unsaturated fatty acids as well as unsaturated fatty acids in rpCholine group without affecting polyunsaturated fatty acids (Table 5). Dietary supplementation did not affect MUN level, while choline content was increased ($P < 0.01$) in milk of rpCholine fed cows (Table 4). Balanced supply of amino acids through MP is necessary for high-yielding cows during early lactation [32]. Additionally, choline is considered to be a critical nutrient during transition and early lactation [16, 21, 24]. Many researchers demonstrated significant improvement in milk and milk component yields upon rpMetLys [26] and rpCholine [24] supplementation. Improvement in milk yield as high as 4.0 kg/day in early lactating Holstein cows supplemented with 40 g rpMet and 700 g of calcium salts of palm fatty acids was reported [3]. In addition, Nam et al. [19] also observed higher milk yield (33.29 vs. 30.52 kg/day; $P < 0.001$) in mid lactating Holsteins fed with 50 g of rpLys and rpMet, in 3:1 ratio, respectively. Furthermore, it is well known that a higher amino acid supply to mammary gland directly enhances milk protein synthesis and volume [7] justifying the results of rpMetLys group. With respect to rpCholine, Lima et al. [16] found a tendency towards increasing milk yield (28.7 vs. 27.9 kg/day; $P = 0.07$) of primigravid Holsteins after supplementation of 15 g of rpCholine/day in the first 80 days of lactation. Higher milk yield upon rpCholine supplementation once again confirms earlier supposition that choline is an essential nutrient during early lactation [24], even in tropical crossbred cows.

Table 2 Characteristics of supplements used in the experiment

Particular	rpMet	rpLys	rpCholine
Methionine (g/kg DM)	526.8 ± 0.4	–	–
Lysine (g/kg DM)	–	402.0 ± 4.6	–
Choline (g/kg DM)	–	–	254.4 ± 2.7
Fat (g/kg DM)	449.0 ± 3.2	490.0 ± 5.0	739.5 ± 4.0
Rumen escape potential (g/kg DM)			
0 h	7.7 ± 2.8	153.4 ± 8.8	168.0 ± 4.9
3 h	202.5 ± 9.1	446.4 ± 5.2	248.4 ± 2.1
6 h	220.1 ± 6.0	464.1 ± 1.6	269.1 ± 3.2
12 h	283.7 ± 6.8	480.4 ± 2.5	294.3 ± 0.8
24 h	441.7 ± 22.8	489.9 ± 1.6	308.3 ± 1.3
Effective degradability	248.0 ± 5.7	450.3 ± 2.2	271.1 ± 0.3
Rumen escape potential	752.0 ± 10.4	549.7 ± 7.9	728.9 ± 8.5

Table 3 Changes in body weight, body condition score and nutrient intake among three groups of crossbred cows

Particular	Control	rpMetLys	rpCholine
Initial body weight (kg)	459.2 ± 20.3	442.7 ± 11.5	416.9 ± 23.78
Final body weight (kg)	440.6 ± 20.6	448.4 ± 12.4	435.5 ± 26.4
Body weight change (kg)	-18.6^{A} ± 6.3	$+5.7^{B}$ ± 5.1	$+18.5^{C}$ ± 3.9
Body condition score	2.95^{a} ± 0.08	3.09^{b} ± 0.06	2.99^{ab} ± 0.07
Nutrient intake (kg/day)			
Dry matter	13.01^{a} ± 0.28	13.36^{b} ± 0.34	13.17^{ab} ± 0.84
Crude protein	2.11^{a} ± 0.10	2.17^{b} ± 0.07	2.02^{ab} ± 0.17
Rumen degradable protein	1.40^{a} ± 0.07	1.43^{b} ± 0.05	1.38^{ac} ± 0.08
Undegradable dietary protein	0.72^{a} ± 0.04	0.74^{b} ± 0.03	0.73^{ab} ± 0.04
Metabolisable protein	1.31^{a} ± 0.06	1.35^{b} ± 0.05	1.33^{ab} ± 0.07
Metabolisable energy (MJ/day)	135.27^{A} ± 2.44	139.37^{B} ± 2.12	135.69^{AC} ± 3.81

Figures with different superscripts in a row differ: a, b, c ($P < 0.01$) A, B, C ($P < 0.05$)

Table 4 Fortnightly production and composition of milk, and nutrient efficiency among three groups of crossbred cows

Particular	Control	rpMetLys	rpCholine
Overall production (kg/day)			
Milk	$15.89^{a} \pm 0.26$	$17.69^{b} \pm 0.19$	$19.28^{c} \pm 1.47$
4 % Fat-corrected milk	$16.21^{a} \pm 1.59$	$18.24^{b} \pm 1.13$	$20.14^{c} \pm 1.43$
Fat	$657.18^{a} \pm 65.38$	$745.32^{b} \pm 46.29$	$828.20^{c} \pm 58.12$
Protein	$518.68^{a} \pm 50.30$	$578.63^{b} \pm 35.43$	$638.56^{c} \pm 46.22$
Lactose	$774.60^{a} \pm 74.83$	$858.08^{b} \pm 53.95$	$939.44^{c} \pm 68.43$
Composition (g/100 g)			
Fat	$4.13^{a} \pm 0.05$	$4.22^{b} \pm 0.05$	$4.30^{bc} \pm 0.03$
Protein	$3.27^{a} \pm 0.03$	$3.28^{ab} \pm 0.02$	$3.31^{c} \pm 0.02$
Lactose	$4.88^{a} \pm 0.01$	$4.86^{b} \pm 0.02$	$4.87^{ac} \pm 0.01$
Milk urea nitrogen (mg/dL)	18.57 ± 0.65	18.44 ± 0.55	17.72 ± 0.58
Milk choline (mg/dL)	$87.69^{a} \pm 2.67$	$88.63^{a} \pm 2.51$	$136.47^{b} \pm 2.84$
Nutrient efficiency			
DM intake[a] (kg):FCM[b] (kg)	$0.80^{a} \pm 0.02$	$0.73^{b} \pm 0.02$	$0.66^{c} \pm 0.02$
CP intake[c] (kg):FCM (kg)	$130.18^{a} \pm 1.75$	$128.99^{b} \pm 1.76$	$100.68^{c} \pm 3.77$
ME intake[d] (Mcal):FCM (kg)	$2.00^{a} \pm 0.03$	$1.83^{b} \pm 0.03$	$1.61^{c} \pm 0.03$

Figures with different superscripts (a, b, c) in a row differ at $P < 0.01$

DM dry matter, *CP* crude protein, *ME* metabolisable energy, *FCM* 4% Fat-corrected milk

Table 5 Milk fatty acid profile (g/100 g total fatty acids) of milk fat among three groups of crossbred cows

Fatty acid	Control	rpMetLys	rpCholine
Caproic acid (C6:0)	1.04 ± 0.03	1.13 ± 0.05	1.06 ± 0.02
Caprylic acid (C8:0)	$2.85^{a} \pm 0.10$	$3.05^{ab} \pm 0.06$	$3.27^{c} \pm 0.09$
Capric acid (C10:0)	3.46 ± 0.16	3.62 ± 0.07	3.54 ± 0.06
Lauric acid (C12:0)	$3.59^{a} \pm 0.04$	$3.42^{b} \pm 0.06$	$3.27^{c} \pm 0.12$
Myristic acid (C14:0)	$10.14^{a} \pm 0.14$	$9.93^{ab} \pm 0.15$	$9.37^{c} \pm 0.06$
Myristoleic acid (C14:1)	$1.42^{a} \pm 0.23$	$1.72^{ab} \pm 0.26$	$2.75^{c} \pm 0.44$
Palmitic acid (C16:0)	27.40 ± 0.36	27.54 ± 0.40	26.40 ± 0.32
Palmitoleic acid (C16:1)	1.34 ± 0.08	1.36 ± 0.05	1.40 ± 0.05
Heptadecanoic acid (C17:0)	0.60 ± 0.05	0.54 ± 0.02	0.52 ± 0.02
Stearic acid (C18:0)	13.93 ± 0.12	13.54 ± 0.24	13.57 ± 0.09
Oleic acid (C18:1*cis* 9)	22.07 ± 0.38	22.39 ± 0.48	22.88 ± 0.16
Elaidic acid (C18:1*trans* 9)	2.04 ± 0.05	2.04 ± 0.04	2.08 ± 0.06
Linoleic acid (C18:2)	$1.88^{a} \pm 0.04$	$1.81^{ab} \pm 0.03$	$1.68^{c} \pm 0.03$
α-linolenic acid (C18:3)	0.77 ± 0.23	0.80 ± 0.23	0.88 ± 0.03
Arachidic acid (C20:0)	0.46 ± 0.02	0.43 ± 0.02	0.43 ± 0.02
∑SFA	$68.83^{a} \pm 0.44$	$68.33^{ab} \pm 0.52$	$66.71^{c} \pm 0.46$
∑MUFA	$28.21^{a} \pm 0.35$	$28.70^{ab} \pm 0.48$	$30.40^{c} \pm 0.44$
∑PUFA	3.09 ± 0.22	2.97 ± 0.24	2.95 ± 0.18
∑UFA	$31.30^{a} \pm 0.46$	$31.67^{ab} \pm 0.52$	$33.35^{c} \pm 0.46$
∑UFA/∑SFA	$0.46^{a} \pm 0.01$	$0.46^{ab} \pm 0.01$	$0.50^{c} \pm 0.01$

Figures with different superscripts (a, b, c) in a row differ at $P < 0.01$

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *UFA* unsaturated fatty acids

Fatty acid profile was not affected due to higher post-ruminal supply of rpMetLys in our study, which corroborates with earlier research findings [15, 27, 35]. Nevertheless, reduction in milk saturated fatty acids and proportional elevation of unsaturated fatty acids in rpCholine group are in line with those of Garg et al. [10],

who supplemented 10 g of rpCholine along with 100 g of bypass fat in crossbred cows. This could be due to the increased supply of preformed fatty acids originating from adipose tissues in the form of VLDL, whose concentration was elevated in rpCholine group.

Nutrient efficiency expressed as ratio of intake of DM, CP and ME to 4 % FCM was different among the groups, and was higher ($P < 0.01$) in both the supplemental groups than that of Control (Table 4). This infers that cows of supplemental groups could utilise the available nutrients towards milk production more efficiently [3]. As efficiency of utilisation of nutrients for milk production is an important determinant affecting farm economy, supplementation of rumen-protected nutrients in the present study demonstrated clear positive benefits among rpMetLys and rpCholine groups.

Table 6 Plasma metabolites among three groups of crossbred cows

Particular	Control	rpMetLys	rpCholine
Glucose (mg/dL)	55.61 ± 1.10	55.07 ± 0.92	56.47 ± 0.53
Phosphatidyl choline (μg/mL)	138.57^{a} ± 3.72	140.98^{ab} ± 3.83	158.92^{c} ± 2.13
Non-esterified fatty acids (mg/L)	106.80 ± 3.46	105.77 ± 1.83	106.35 ± 1.47
Triglycerides (mg/dL)	13.40^{a} ± 0.34	16.22^{b} ± 0.54	14.84^{c} ± 0.29
Very low density lipoproteins (mg/dL)	2.68^{a} ± 0.11	3.24^{b} ± 0.06	2.97^{c} ± 0.06
Vitamin E (μg/mL)	1.01^{a} ± 0.07	0.90^{ab} ± 0.004	1.13^{c} ± 0.08
Cholesterol (mg/dL)	193.16 ± 6.93	198.31 ± 5.94	198.63 ± 6.61
Plasma urea nitrogen (mg/dL)	18.13 ± 0.52	18.01 ± 0.53	17.86 ± 0.53
Lysine (μmol/dL)	13.91^{a} ± 1.03	15.08^{c} ± 0.93	13.94^{ab} ± 0.45
Methionine (μmol/dL)	5.71^{a} ± 0.38	6.05^{bc} ± 0.19	6.06^{c} ± 0.27

Figures with different superscripts (a, b, c) in a row differ at $P < 0.01$

Plasma Metabolites and Amino Acid Profile

Plasma metabolites like glucose, NEFA, cholesterol and PUN were similar among all groups. Concentration of triglycerides and VLDL was lower ($P < 0.01$) in rpMetLys and rpCholine than Control. However, phosphatidylcholine and vitamin E levels were higher ($P < 0.01$) in rpCholine than other two groups. Lysine levels were higher ($P < 0.01$) for both treatment groups, whereas methionine was increased ($P < 0.01$) only in rpMetLys.

Levels of various plasma metabolites reflect nutritional and metabolic status of cows during early lactation. Glucose concentration among all the groups was within normal physiological range and was not altered by dietary treatments. This could be due to a high metabolic rate of utilisation of glucose as well as homeostatic mechanism, which did not allow appreciable changes in glucose level [31, 38]. NEFA, an indicator of energy status and fat mobilisation during early lactation, was also similar in all animals. Furthermore, there was no difference in cholesterol and PUN levels among the groups, and were within normal range [10, 31]. As expected, phosphatidyl choline concentration was increased in rpCholine group, which could be due to metabolic conversion of phosphatidylethanolamine into phosphatidylcholine [24]. Higher plasma triglycerides observed in rpCholine group are in accordance with the observations of Bindel et al. [5], who reported that supplemental rpCholine led to increase ($P < 0.10$) in plasma triglycerides as a result of greater hepatic export [13], while the reasons for elevated triglycerides upon rpMetLys are not known. Supplemental rpMetLys and rpCholine facilitate hepatic lipoprotein synthesis, thereby increasing plasma VLDL concentrations [9, 28]. Higher vitamin E levels observed in rpMetLys group concur with the earlier report of Pinotti et al. [25], in which it was postulated that choline availability influences vitamin E status in dairy cows, although actual mechanism has not been elucidated with certainty. As plasma amino acid concentrations increase only when supply exceeds the requirement [20], it is reasonable that the daily dose of rpMet (5 g) and rpLys (20 g) used in this study completely fulfilled these amino acid requirements, as revealed by their corresponding increased levels in plasma. Additionally, the metabolic pathways involving methyl donor

Table 7 Reproductive performance among three groups of crossbred cows

Particular	Control	rpMetLys	rpCholine
Commencement of cyclicity (days)	65.13 ± 2.26	64.11 ± 1.02	62.44 ± 1.42
Days open (days)	164.2^{A} ± 9.53	152.4^{AB} ± 6.64	131.3^{C} ± 19.25
Artificial insemination/conception	2.60 ± 0.81	2.67 ± 0.51	2.29 ± 0.76
Conception rate (%) during 120 days of experiment	55.6	66.7	77.8

Figures with different superscripts (A, B, C) in a row differ at $P < 0.05$

functions of choline and methionine are inter-related [24]. There might have been a sparing effect of methyl group of methionine, which was not utilised for choline synthesis, thus leading to elevated plasma methionine concentration in rpCholine group (Table 6).

Reproductive Performance

The time required for commencement of cyclicity was similar among three groups (Table 7). Days open was decreased ($P < 0.01$) in animals belonging to rpCholine group. Number of AI per conception and conception rates did not differ due to treatments. The commencement of cyclicity is related with the process of involution of uterus. The days open (service period) was shorter in rpCholine group than that of other two groups, suggesting that lesser time was required for conception in rpCholine supplemented cows. As cows in both the supplemental groups had desired BCS, they expressed post-partum estrus relatively earlier than cows in Control group.

Conclusions

It was concluded from the present study that supplementation of 5 g of rpMet plus 20 g of rpLys and 50 g of rpCholine during late gestation and 4 months of lactation improved production of milk and milk components. Furthermore, these supplements improved metabolic health status and BCS leading to better reproductive performance. Thus, this study establishes that the supplementation of rpMetLys and rpCholine to high-yielding crossbred cows was beneficial under Indian feeding systems.

Acknowledgments Authors thankfully acknowledge the Director, ICAR-National Dairy Research Institute, Karnal for rendering necessary research facilities to complete this study.

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