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Perfromance of Lactating Dairy Cows in Response to Supplementation of Rumen-Protected Choline

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Abstract: Effects of rumen-protected choline supplementation on milk production and milk fatty acids in crossbred Holstein Friesian dairy cows were studied. Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 32±8 days in milk, 16.0±1.6 kg of milk and 426±27 kg body weight were blocked by milking days first and then stratified balanced milk yield and body weight into three groups of 8 cows. The first group (Control) received approximately 9 kg of concentrate. The second group was fed the same basal diet as the control group and supplemented with 20 g day^{-1} of Rumen-Protected Choline (RPC) and the third group was fed the same basal diet as the control group and supplemented with 40 g day^{-1} of RPC. All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*, 55 days cutting interval) had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 10 weeks with the 1st 2 weeks being considered as adaptation period and measurements were made during the last 8 weeks. Daily milk yields were recorded. Milk sample and dry matter intake were collected in 2 consecutive days weekly. Live weights were recorded at the start and at the end of the experiment. Milk choline and blood parameters were also analyzed. The results showed no statistical significant differences in intakes, live weight change, milk compositions and blood parameters ($p>0.05$) however, milk yield, 3.5% fat-corrected-milk yield and milk choline were increased by rumen-protected choline supplementation. It is recommended in the present study that the addition of 20 g day^{-1} rumen-protected choline could be beneficial to lactating dairy cows in early lactation.

Key words: Rumen-protected choline, milk production, milk composition, milk choline, blood parameters, Thailand

INTRODUCTION

The transition period is a time of rapid change in metabolism of lactating dairy cow. The onset of milk production increases the demand for glucose and amino acids. Simultaneously, daily nutrient intake is insufficient to meet the requirement for milk production and energy balance is negative (Van Saun, 1991; Bell *et al*., 1995). Due to decreased feed intake at the end of gestation, the period of negative energy balance often starts prior to calving (Van den Top *et al*., 1995). The level of Non-Esterified Fatty Acids (NEFA) increases in plasma as a consequence of body fat mobilization (Ford, 1959; Reid and Collins, 1980) and leads to hepatic lipidosis. Fatty liver is a metabolic disorder and high liver fat content adversely affects health (Hill *et al*., 1985), depresses milk production and is associated with reproductive problems (Reid, 1982; Gerloff *et al*., 1986). Earlier studies (Reid *et al*., 1979; Smith *et al*., 1997) proved that the major cause of fat accumulation was impaired triglyceride output from the liver resulting from the decreased Very Low Density Lipoprotein (VLDL) secretion. Choline, a component of phospholipid and methyl donor, plays an essential role in VLDL synthesis and thereby contributes to fat export from the liver. Earlier studies (Piepenbrink and Overton, 2000; Pinotti *et al*., 2002; Cooke *et al*., 2007) suggested that high-producing cows may be choline deficient around parturition which adversely affects liver functions especially the synthesis and secretion of VLDL. Higher choline supply may increase milk production (Erdman and Sharma, 1991; Hartwell *et al*., 2000; Pinotti *et al*., 2003) but this response is strongly influenced by other nutrients such as protein and methionine (Emmanuel and Kennelly, 1984; Hartwell *et al*., 2000; Brusemeister and Sudekum, 2006). However, other researchers did not find any difference in milk yield of the cows in response to choline supplementation (Piepenbrink and Overton, 2003;

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Guretzky *et al*., 2006). Dietary choline is degraded rapidly by the rumen microorganisms (Neill *et al*., 1979; Sharma and Erdman, 1989) hence, supplementation with choline (Conveniently as its salt, choline-chloride) is not an effective way to increase choline supply. Therefore, rumen-protected forms of choline have been developed to deliver choline to the small intestine for absorption. The aim of the present study was to determine the effect of Rumen-Protected Choline (RPC) supplementation on performances and blood parameters of lactating dairy cows.

MATERIALS AND METHODS

Animals and treatments: Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 32±8 days in milk, 16.0±1.6 kg of milk and 426±27 kg body weight were blocked by milking days first and then stratified balanced milk yield and body weight into three groups of 8 cows. The first group (Control) received approximately 9 kg of concentrate. The second group was fed the same basal diet as the control group and supplemented with 20 g day^{-1} of Rumen-Protected Choline (RPC) and the third group was fed the same basal diet as the control group and supplemented with 40 g day^{-1} of RPC (Reashure®, Balchem Co., Ltd.). Reashure® contains 25% choline in a chemical form of choline-chloride hence, Reashure® fed at 80 and 160 g day^{-1} to provide 20 and 40 g day^{-1} of choline, respectively.

All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*, 55 days cutting interval) had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 10 weeks with the 1st 2 weeks as the adjustment period followed by 8 weeks of measurement period.

Measurements, sample collection and chemical analysis: Feeds offered and residues left after eating of individual cows were weighed for 2 consecutive days of each period and samples were taken and dried at 60°C for 48 h. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis. Samples were ground through a 1 mm screen and subjected to proximate analysis. The crude protein content was determined by Kjeldahl analysis (AOAC, 1998). Ether extract was determined using petroleum ether in a Soxtec system (AOAC, 1998). Neutral detergent fiber and acid detergent fiber were determined using the method described by Van Soest *et al.* (1991) adapted for Fiber analyzer. Chemical analysis was expressed on the basis of the final DM. Cows were milked twice daily at 05:00 and 15:00 h and milk yields were recorded for each cow. Samples of milk (Evening+morning) were collected at each milking for 2 consecutive days weekly and stored at 4°C with a preservative (Bronopol tablet; D and F Control system, San Ramon, CA) until analyzed for fat, protein, lactose and solid-not-fat contents using a Milko-Scan S50 analyzer (Tecator, Denmark). All cows were weighed at the start and end of the experiment.

Milk choline analysis: On day 50th, milk sample was collected from individual cow, freeze-dried and stored frozen at -20°C for milk choline analysis. Milk choline was determined by the enzymatic method of Woollard and Indyk (2000). Briefly, 5 g of freeze-dried sample was digested by 30 mL of 1.0 M hydrochloric acid at 70°C for 3 h to release the majority of bound choline. After cooling, pH was adjusted with 50% NaOH to 3.5-4.0. The hydrolysate was diluted to 50 mL with water and filtered. The residual choline from phospholipids was cleaved with phospholipase D (Sigma Type VI, P-8023 from Streptomyces chromofuscus, 150 unit mg^{-1}, unit definition: 1 unit liberates 1.0 mmol choline from L-a-phosphatidyl choline h^{-1} at pH 5.0 at 30°C; Sigma-Aldrich, St Louis, USA).

Free choline reacted with choline oxidase (Sigma C-5896 from *Alcaligenes* species, 10 unit mg^{-1}, unit definition: 1 unit forms 1.0 mmol H_2O_2 with oxidation of 1 mmol choline to betaine aldehyde min^{-1} at pH 8.0 at 37°C; Sigma-Aldrich) liberating hydrogen peroxide. In the presence of peroxidase (Sigma type I, P-8125 from horseradish, 80 unit mg^{-1}, unit definition: 1 unit forms 1.0 mg purpurogallin from pyrogallol in 20 sec at pH 6.0 at 20°C; Sigma-Aldrich), phenol is oxidized, forming a chromophore with 4-aminoantipyrine (Sigma A-4382; Sigma-Aldrich).

Absorbance of this compound was measured at 505 nm. Choline level was calculated as choline hydroxide by the mean of a standard solution prepared by dissolving 523 mg of choline bitartarate (Sigma C-2654; Sigma-Aldrich) in 100 mL of water which was equal to 2500 mg mL^{-1} choline hydroxide solution. The five point standard curve (50, 100, 150, 200 and 250 mg mL^{-1} choline hydroxide equivalent) was prepared by further diluting the standard solution in water.

This method measures the total choline in milk: Free choline plus choline bound as acetylcholine, phosphatidylcholine, lysophosphatidylcholine, sphingomyelin and glycerophosphocholine.

Plasma analysis: Jugular vein blood samples were taken before the first feed of the day, on day 50 postpartum. The samples were collected into heparinized tubes (Venoject®; Terumo Europe, Leuven, Belgium) and centrifuged (14, 000 g for 15 min at 10°C) to obtain plasma

which was stored at -20°C, until analysis for glucose (Sigma Chemical Co., St Louis, MO, USA), Non-Esterified Fatty Acids 4 (NEFA) (Enzycolor, Japan), cholesterol (Siegel and Bowdoin, 1971) and β-hydroxybutyrate (Sigma Chemical Co., St Louis, MO, USA).

Statistical analysis: Measurements of intake, milk production, milk composition, milk choline and blood parameters were analyzed by ANOVA for a randomized complete block design using the Statistical Analysis System (SAS). Differences between treatment means were statistically compared using least significant differences.

RESULTS AND DISCUSSION

Chemical composition, estimated energy values and degradability of Dry Matter (DM) and Crude Protein (CP) of feeds used in the experiment are shown in Table 1. The crude fat content and energy values of grass silage were low. This is probably because forage was harvested at a more mature stage (55 days cutting age) and consequently, resulting in low DM and CP degradability (47.9 and 52.6%, respectively). DM, CP and net energy for lactation (NE_{LP}) intakes of the experimental cows were similar ($p>0.05$) (Table 2). The similar DMI of the control and RPC-supplemented cows is in agreement with several earlier studies (Erdman and Sharma, 1991; Hartwell *et al*., 2000; Piepenbrink and Overton, 2003; Pinotti *et al*., 2003; Zahra *et al*., 2006).

The significant increases in milk yield and 3.5% FCM yield in RPC treated cows of the present experiment are in line with the observations of Erdman and Sharma (1991) and Pinotti *et al*. (2003) however, Piepenbrink and Overton (2003) reported no significant milk yield response when cows received RPC supplementation.

Milk fat concentration was not altered by RPC treatment in this and in several earlier experiments (Bauchart *et al*., 1998; Deuchler *et al*., 1998). However, Erdman and Sharma (1991) reported a quadratic response of milk fat concentration to RPC with a decrease when RPC was added at 0.78 g kg^{-1} of DM and increases at 1.56 and 2.34 g kg^{-1} RPC supplementations. In the present experiment, 20 and 40 g day^{-1} RPC numerical increased fat yield by 9.2 and 12.0%, respectively. Similar to earlier trials, RPC increased fat yield by 7% as an effect of higher milk yield. Piepenbrink and Overton (2003) and Pinotti *et al*. (2003), respectively reported 8.2 and 20.0% improvement in fat yield in the RPC-supplemented group. Theoretically, choline contributes to fatty acid transport in blood and it may enhance the availability of fatty acids for milk fat synthesis. Choline is incorporated also in phospholipid membranes around fat globules hence, higher choline supply may increase fat yield (Table 3).

Table 1: Chemical composition of concentrate and grass silage used in the experiment

Dry matter (%)	Concentrate	Grass silage
Dry matter	92.27±0.04	29.25±0.06
Crude protein	21.43±0.15	5.74±0.03
Crude fat	4.16±0.13	1.74±0.11
Ash	8.52±0.04	10.05±0.98
Crude fiber	12.38±0.11	34.33±0.27
Neutral detergent fiber	38.29±0.21	68.28±0.32
Acid detergent fiber	16.76±0.19	50.91±0.27
Acid detergent lignin	3.67±0.16	4.62±0.09
Neutral detergent insoluble nitrogen	1.14±0.01	0.27±0.02
Acid detergent insoluble nitrogen	0.42±0.01	0.13±0.01
TDN_{1X} (%)[1]	70.19	53.71
DE_P (Mcal kg^{-1})[2]	3.01	2.41
ME_P (Mcal kg^{-1})[3]	2.60	1.97
NE_{LP} (Mcal kg^{-1})[4]	1.64	1.19
dgDM	67.50	47.90
dgCP	66.10	52.60

dgDM = Effective degradability of Dry Matter, dgCP = Effective degradability of Crude Protein; TDN_{1X} (%) = tdNFC + tdCP + (tdFA×25.25) + tdNDF−7); DE_{1X} (Mcal kg^{-1}) = ((tdNFC/100)×4.2) + ((tdNDF/100)×4.2)×((tdCP/100)×5.6) + ((FA/100)×9.4)−0.3; [2]DE_P (Mcal kg^{-1}) = ((TDN_{1X}−((0.18×TDN_{1X})−10.3))×Intake)/TDN_{1X})×DE_{1X}; [3]ME_P (Mcal kg^{-1}) = (1.01×(DE_P)−0.45) + (0.0046×(EE-3)); [4]NE_{LP} (Mcal kg^{-1}) = (0.703×ME_P)−0.19, (EE>3%); NE_{LP} (Mcal kg^{-1}) = (0.703×ME_P)−0.19) + ((0.097×ME_P)/97)×(EE−30), (EE>3%)

Table 2: Effect of rumen-protected choline supplementation on intakes

Intake	Control	20 g RPC	40 g RPC	SEM	p-value
DM intake (kg day^{-1})					
Concentrate	8.30	8.30	8.30	-	-
Grass silage	6.08	6.26	6.13	0.24	0.538
Total	14.38	14.56	14.43	0.26	0.521
CP intake (g day^{-1})					
Concentrate	1779.00	1779.00	1779.00	-	-
Grass silage	349.00	359.00	352.00	16.26	0.552
Total	2128.00	2138.00	2131.00	16.76	0.506
NE_L intake (Mcal day^{-1})					
Concentrate	13.62	13.62	13.62	-	-
Grass silage	7.25	7.46	7.31	0.31	0.569
Total	20.87	21.08	20.95	0.30	0.533

RPC = Rumen Protected Choline; SEM = Standard Error of the Mean

Table 3: Effect of rumen-protected choline supplementation on milk yield, milk composition, live weight and live weight change

Parameters	Control	20 g RPC	40 g RPC	SEM	p-value
Milk yield (kg day^{-1})	15.80[b]	16.60[ab]	16.80[a]	0.31	0.048
3.5% fat corrected milk (kg day^{-1})	17.00[b]	18.30[ab]	18.60[a]	0.30	0.047
Fat yield (g day^{-1})	626.00	684.00	701.00	15.48	0.542
Protein yield (g day^{-1})	450.00	483.00	514.00	11.34	0.771
Lactose yield (g day^{-1})	758.00	808.00	822.00	11.18	0.528
Solid not fat yield (g day^{-1})	1319.00	1408.00	1453.00	22.45	0.463
Total solid yield (g day^{-1})	1945.00	2092.00	2154.00	33.28	0.461
Fat (%)	3.96	4.12	4.17	0.12	0.583
Protein (%)	2.85	2.91	3.06	0.09	0.907
Lactose (%)	4.80	4.87	4.89	0.04	0.901
Solid not fat (%)	8.25	8.48	8.65	0.15	0.823
Total solid (%)	12.31	12.60	12.82	0.24	0.629
Initial weight (kg)	385.00	384.00	387.00	10.00	0.842
Final weight (kg)	406.00	409.00	417.00	11.00	0.394
Live weight change (g day^{-1})	+375.00	+446.00	+536.00	125.00	0.918

RPC = Rumen-Protected Choline; SEM = Standard Error of the Mean; Means within a row with different superscript differ ($p<0.05$)

Table 4: Effect of rumen-protected choline supplementation on milk choline and blood parameters

Parameters	Control	20 g RPC	40 g RPC	SEM	p-value
Milk choline (mg kg^{-1})	99.66^{b}	138.50^{a}	143.20^{a}	5.68	0.036
Plasma glucose (mmol L^{-1})	4.29	4.40	4.43	0.08	0.756
Plasma BHBA (mmol L^{-1})	0.55	0.51	0.52	0.06	0.923
Plasma NEFA (mmol L^{-1})	0.60	0.54	0.56	0.05	0.873
Plasma cholesterol (mmol L^{-1})	3.75	4.12	4.06	0.13	0.764
NEFA/Cholesterol	0.16	0.13	0.14	0.02	0.812

RPC = Rumen-Protected Choline; BHBA = β-Hydroxyl Butyrate; NEFA = Non-Esterified Fatty Acids; SEM = Standard Error of the Mean; Means within a row with different superscript differ ($p<0.05$)

Table 5: Energy supply and energy requirement (Mcal day^{-1})

Energy supply and requirements	Control	20 g RPC	40 g RPC	SEM	p-value
Net Energy intake (NE_{LP} intake)	20.87	21.38	21.55	0.31	0.568
Net Energy for maintenance (NE_{LM})	7.75	7.76	7.85	0.16	0.837
Net Energy for lactation (NE_{LL})	11.27	12.19	12.57	0.19	0.671
Net Energy for gain (NE_{LG})	1.03	1.25	1.55	0.24	0.842
Net Energy retention (NE_{LR})	18.73^{b}	19.86^{ab}	20.57^{a}	0.38	0.039
Efficiency of energy utilization	0.90	0.94	0.98	0.03	0.446

RPC = Rumen-Protected Choline; SEM = Standard Error of the Mean; NE_{LP} = Net Energy for Lactation at Production level; NE_{LM} = Net Energy requirement for Maintenance = $0.08\times LW^{0.75}$; NE_{LG} = Net Energy requirement for Gain = Reserve energy×(0.64/0.75) reserve energy = NRC (2001); NE_{LL} = Net Energy requirement for Lactation = Milk yield (kg day^{-1})×(0.0929×% fat 0.0547×% CP+0.0395×% Lactose); NE_{LR} = Net Energy retention; Efficiency of energy utilization = NE_{LR}/NE_{LP} intake

Milk protein concentration and protein yield were not affected by RPC supplementation in earlier studies (Hartwell *et al*., 2000; Piepenbrink and Overton, 2003; Pinotti *et al*., 2003). In these experiments, RPC was administered in a lower amount (12-20 g day^{-1}) than in ours. However, Erdman and Sharma (1991) measured a quadratic relationship of milk protein content with increasing dietary RPC concentration. In this experiment, 7.3 and 14.2% improvement of protein yield were detected. Choline is a source of methyl groups and acts also as a methyl donor in transmethylation reactions. In this function, choline and methionine metabolism are closely related. Emmanuel and Kennelly (1984) found that 28% of the absorbed methionine is used for choline synthesis in lactating goats. The first limiting amino acid on corn, soybean meal, corn silage and alfalfa-based diets is usually methionine (Schwab *et al*., 1992; Rulquin *et al*., 1993). Armentano *et al*. (1997) supplemented the diet of lactating cows with 10.5 g day^{-1} rumen-protected methionine and found a 1 g kg^{-1} increase in milk protein concentration and a 42 g increase in protein yield although, no milk yield response was reported. In this trial, methionine was the first limiting amino acid. The calculated concentration of digestible methionine in the metabolizable protein was 18.1 g kg^{-1} which was slightly below the recommendation of NRC (2001) might be resulting in a limited milk protein synthesis. Additional RPC may decrease the utilization of methionine for choline synthesis; consequently, more methionine is available to support milk protein synthesis in the mammary gland.

Milk choline concentration increased for both supplemented groups (Table 4). A similar increase in milk-free choline was found by Newbold *et al*. (2005). The diet in their experiment did not contain any choline supplementation and the milk choline concentration and yield were measured over the period from 15 until 90 DIM. The researchers reported an 82% increase in milk choline concentration and a 117% increase in choline yield between 15 and 30 DIM. Bitman and Wood (1990) studied the concentration of phospholipids in milk on days 3, 7, 42 and 180 of lactation. An increase was reported between 3 and 7 days but a steady decline was observed between 7 and 180 DIM which might be a consequence of significantly decreasing milk fat concentration. The phospholipid fraction of milk fat was continuously increased during the 1st 42 days of lactation but free choline unfortunately was not measured in their experiment. The RPC-supplemented group showed higher milk choline concentration than the control group. The higher milk choline level provides evidence that choline in the experimental RPC product escaped ruminal fermentation, absorbed from the small intestine and improved the choline supply of the cows.

Rumen-protected choline supplementation had no effect on plasma levels of glucose (4.29 vs 4.40 and 4.43 mmol L^{-1} in control, 20 and 40 g RPC animals), β-hydroxybutyrate (0.55 vs. 0.51 and 0.52 mmol L^{-1}), cholesterol (3.75 vs. 4.12 and 4.06 mmol L^{-1}), NEFA (0.60 vs. 0.54 and 0.56 mmol L^{-1}) or the NEFA/cholesterol ratio (0.16 vs. 0.13 and 0.14) (Table 4).

When combining the data for milk yield and Body Weight (BW) change, it was possible to compare the effect of different rations on the apparent utilization of the Net Energy for Lactation at Production (NE_{LP}) intake (Table 5). Both groups of cows consumed similar NE_{LP}, therefore the partitioning of energy between milk productions was also similar.

Both groups of cows had a considerable supply of NE_{LP} but the milk yields were lower than would have been expected from NE_{LP} intakes. The respective intakes of 20.9, 21.4 and 21.6 Mcal daily by cows in the control, 20 and 40 g RPC groups in theory should be able to produce approximately 17.6, 17.7 and 17.1 kg of milk/day, respectively. The lower milk yield than what would be expected from the NE_{LP} available could be attributed to the

Table 6: Protein supply from feeds (g day^{-1}) and protein requirement (g day^{-1}) of cows

Parameters	Control	20 g RPC	40 g RPC	SEM	p-value
Rumen Degradable Protein requirement (RDP_{req})	1430	1467	1474	21.4	0.778
Rumen Degradable Protein supply (RDP_{sup})	1359	1365	1361	6.5	0.819
Deficit/surplus	-71	-102	-113	16.1	0.652
Metabolizable Protein requirement (MP_R)	1074	1113	1134	31.2	0.917
Rumen Undegradable Protein requirement (RUP_{req})	432	463	496	51.2	0.761
Rumen Undegradable Protein supply (RUP_{sup})	486	488	486	6.6	0.926
Deficit/surplus	+54	+25	-10	32.3	0.613

RPC = Rumen-Protected Choline; SEM = Standard Error of the Mean; RDP_{req} = Rumen Degradable Protein requirement = 0.15294×TDN actual; RDP_{sup} = Rumen Degradable Protein supply = Total DM fed×1000×diet CP×CP_RDP; RUP_{req} = Rumen Undegradable Protein requirement = Total CPReq-(MP Bact+MP Endo)/diet RUPDigest; RUP_{sup} = Rumen Undegradable Protein supply = CP Total-RDP_{sup}

probable underestimates of the net energy for lactation at maintenance (NE_{LM}) for dairy cows in the tropics. Since, dairy cows in the tropics are fed lower quality feeds than cows in the United States, the use of the equation suggested by the NRC (2001) might be inappropriate. AAC (1990) recommended that dairy cattle consuming feeds containing energy lower than 10 MJ Metabolizable Energy (ME) kg^{-1} DM needed more energy for maintenance. The present study used a net energy maintenance value of 0.08 Mcal kg^{-1} $BW^{0.75}$ for predicting NE_{LM}. If the hypothesis by AAC (1990) is true with the assumption that the average net energy values of milk and body weight change are unaffected by the quality of feeds as in case of NE_{LM}, the average net energy maintenance value of 0.082 Mcal kg^{-1} $BW^{0.75}$ should be used in this study. This is approximately 2.5% higher than the NRC (2001) recommendation. Suksombat and Mernkratoke (2004) and Suksombat and Janpanichcharoen (2005) suggested that in the tropics, the average net energy maintenance value of 0.083 and 0.106 Mcal kg^{-1} $BW^{0.75}$, respectively would be more appropriate than the value of 0.08 Mcal kg^{-1} $BW^{0.75}$ recommended by NRC (2001). Before a conclusion can be reached, further research is needed.

The estimated supplies of Rumen Degradable Protein (RDP) and Rumen Undegradable Protein (RUP) to the cows can be calculated using the protein degradability values of each feed (Determined by nylon bag technique; Table 6; NRC, 2001). All cows consumed similar RDP and RUP however, all cows received inadequate RDP but cows on 0 and 20 g RPC consumed adequate RUP while cows on 40 g RPC received inadequate RUP. The deficit in RDP supply relative to demand would have reduced microbial protein synthesis and thus a low quantity of microbial protein would have reached the small intestine.

The present study indicated that during early lactation, approximately 20 g day^{-1} rumen-protected choline supplemented to lactating dairy cows gave a beneficial response.

CONCLUSION

Feeding RPC did not alter intakes, milk composition, live weight change and blood parameters however, milk yield, 3.5% fat-corrected-milk yield and milk choline concentration were increased for both supplemented groups. RPC supplementation significantly increased milk choline concentration, indicating better choline supply to these cows. It is recommended that approximate 20 g day^{-1} of RPC could be added to lactating dairy cow's diet for better beneficial response.

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