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Effect of Dietary Rumen Protected Methionine and/or Choline Supplementation on Rumen Fermentation Characteristics and Productive Performance of Early Lactating Cows

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Abstract: Sixty early lactating Holstein dairy cows were fed for 12 weeks on berseem hay and cereal grains based total mixed ration formulated with the MET content limited (40 g/day) to investigate the effect of supplementing rumen protected methionine (15 g/day) and/or choline (30 g/day) on rumen fermentation characteristics, productive performance and some blood serum parameters. Cows were allotted into four groups and fed on the basal diet without supplementation, supplemented by 15 g/day of RP-MET, 30 g of RP-CHOL or 15 g MET + 30 CHOL respectively. Average body weight was not significantly different among treatment, but cows fed on basal diet supplemented by both RP-MET and RP-CHOL was mobilizing less body tissue in the post-partum period. RP-MET improved ($p<0.05$) DMI by 0.7% when compared with MET limited group, while RP-CHOL significantly improved DMI by 8.4% throughout the whole experimental period when compared with the control group cows. There was an improvement of milk production with RP-MET supplementation by 2.5% across the whole experimental period when compared with MET limited group, while RP-CHOL improved milk production by 5.9 and 3.3% when compared with cows fed on the control basal diet and both MET and CHOL treated diets, respectively. Milk protein yield tended to increase with RP-MET and RP-CHOL supplementation by 5.3 and 7.4% when compared with cows fed on the basal diet without supplement. Both RP-MET and RP-CHOL improved ECM and milk-to-feed ratio but had no significant effect on rumen fermentation characteristics. Rumen protected MET induced no significant effect on blood serum glucose, triglyceride, NEFA and blood urea concentration while reduced ($p<0.05$) blood serum cholesterol when compared with MET limited group. Moreover, RP-CHOL significantly increased blood serum glucose and cholesterol concentration and reduced triglyceride, NEFA and urea concentrations. It could be concluded that Dietary RP-CHOL (30 g/day) to early lactating dairy cows that received a MET limited diet improved DMI, milk yield and increased milk protein yield. In conclusion, supplementing RP-MET (15 g/day) or both RP-MET + RP-CHOL were not beneficial as RP-CHOL supplementation alone.

Key words: Dairy cattle, methionine, choline productive performance, rumen fermentation

INTRODUCTION

Nutritional strategies to increase the yield and content of protein in milk from lactating dairy cows continue to be of substantial economic interest to the dairy industry. Most of the focus in research and industry applications has been directed toward increasing the absorbance of Methionine (MET) and lysine as the first two limiting amino acids in most dietary situation (Bequette *et al*., 1998; NRC, 2001; Doepel *et al*., 2004; Lapierre *et al*., 2006; Benefield *et al*., 2009). The most efficient approach to increase the supply of absorbable MET to dairy cows is to include a rumen protected MET. Methioine metabolism is closely linked to that choline and it is important in the dairy cow because it is required for milk protein synthesis. MET is, also, involved in many pathways including the synthesis of phospholipids, carnitine, creatine and polyamines (Bequette *et al*., 1998; Berthiaume *et al*., 2006). In addition, MET is the source of the methyl donor *S*-adenosyl methionine, the metabolite that provides methyl groups in a variety of reactions including the de novo synthesis of choline from phosphatidylethanolamine. In addition, choline increases the supply of methyl groups, which can affect the availability of other methyl donor compounds (Frank and Karl-Heinz, 2006). Moreover, Emmanuel and Kennelly, 1984; Lobley *et al*., 1996; demonstrated that up

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to one third of the total Met supplement can be lost due the need to synthesize choline. Because of these metabolic relationships, dietary supply of choline affects Met requirements and Met supply can affect choline metabolism. Since choline (Erdman *et al*., 1984) is susceptible to rapid ruminal degradation, the amounts available for absorption are limited. Therefore, dairy cows may benefit from Rumen-Protected (RP) supplementation of choline.

Choline also participates, via the compound Phosphatidylcholine, in the removal of triglycerides from the liver by incorporation of triglycerides into lipoproteins (Pinotti *et al*., 2002). Lipotropic compounds have the ability to prevent and subsequent to a deficiency, correct excess fat deposition in the liver (Zeisel, 1992; NRC, 2001).

Feeding RP forms of Met to early lactation dairy cattle has increased milk and milk protein yield (Donkin *et al*., 1989; Davidson *et al*., 2008), as well as milk fat yield (Overton *et al*., 1996). Researchers also have reported that dairy cattle can produce more milk when fed supplemental RP-choline (Erdman and Sharma, 1991; Pinotti *et al*., 2003).

MET (Onodera, 1993) and choline (Atkins *et al*., 1988) are degraded by microorganisms in the rumen, so Rumen Protected (RP) forms are more effective at supplying the compounds to the cow than forms that are not protected. There has been extensive research conducted to develop and determine the effectiveness of technologies for protecting MET (Schwab, 1996). However, few researches that investigates the effectiveness of RP choline has been conducted, even though some researchers have studied the effects of free choline (Sharma and Erdman, 1989) on ruminal fermentation in cannulated cows.

The objective of this study was to evaluate the effects of supplementing RP forms of MET and/or Choline (CHOL) to a limited Met diet on the rumen fermentation, lactation performance and some blood parameters of early lactation cows.

MATERIALS AND METHODS

This experiment was carried out to evaluate the effect of limited and high dietary rumen protected methionine without or with rumen protected choline supplementation on rumen fermentation characteristics, productive performance and some blood parameters of early lactation cows.

Cows, experimental design and diets: Sixty early lactating Holstein dairy cows were allotted into four groups (15 cows per each). The first group (15 cows) was assigned as the control group without any supplement and considered as methionine limited group. The other groups were fed on the basal diet supplemented with 0.09% of RP-MET (substituted for ground corn), or 0.6% of RP-Choline (substituted for ground corn) or RP-MET (0.09%) plus RP-CHOL (0.6%), respectively from the first day through week 12 post-partum. These amounts, assuming an average Dry Matter Intake (DMI) of 20 kg/day per cow, should have resulted in intake of 0, 15 g (RP-MET) from "Mepron 85" produced by "Evonik-Degussa Corporation, Kennesaw, GA", Mepron is a rumen slow-release product which contains 85% DL-MET and small amounts of ash, starch, fat and cellulose. This product consists of a nucleus of MET with several thin coats of stearic acid and ethyl cellulose), 30 g RP-CHOL (25% wt/wt of choline; Reashure choline, Balchem Encapsulates, New Hampton, NY) and 15+30 g (RP-MET + RP-CHOL) daily. A berseem hay and cereal grains based Total Mixed Ration (TMR) was formulated to meet the NRC (2001) recommendations for net energy for lactation (NE_L), Metabolizable Protein (MP), Rumen Undegreadable Protein (RUP), Rumen Degradable Protein (RDP), macrominerals, microminerals and the vitamins A, D, and E (Table 1). In addition, the TMR was formulated to contain a limited amount of Met.

Feed intake and body weight: Diets were offered in equal amounts twice daily (08:00 and 15:00). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows. Samples of TMR and feed ingredients were collected daily and kept frozen. Samples were composited by period (each 4 weeks), dried at 55°C for 48 h, ground through a 1-mm screen (Wiley mill) and analyzed for DM, OM, total nitrogen, NDF, ADF, EE composition. Body Weight (BW) was determined one day postpartum and then each 4 weeks after the a.m. milking. Based on that milk- to-feed ratio Energy Corrected Milk (ECM) were calculated monthly (NRC, 2001).

Milk production and milk composition: Cows were milked twice daily in the milking pallor (05:00 and 17:00), and milk yield was recorded at each milking. During the last week of each 28-day period, milk samples were taken from each cow at each milking, pooled on a yield basis and stored at +4°C with a preservative (bronopol-B2) until analyzed for fat and protein contents.

Ruminal fermentation characteristics: Ruminal fluids were collected at 0, 4 and 8 h after feeding from 5 cows from each group on the 28th, 56th and 84th day during the experimental period. Ruminal fluids were collected through a speculum were inserted into the cow mouth, and a lubricated stomach tube was inserted through the speculum into the rumen via the esophagus. Ruminal contents (250 ml) were obtained using an electric pump. Samples were monitored visually to ensure they

Table 1: Ingredients (% as fed basis) and chemical (% on dry matter basis) composition of the diet

Physical composition (as fed basis)		Chemical composition (On dry matter basis)	
Ingredients	%	Items	%
Berseem hay	45.0	Crude protein	17.25
Yellow corn grain	21.0	Ether extract	3.9
Barley grain	16.0	Crude fiber	16.1
Soybean meal (48%)	7.5	NDF	26.8
Whole cotton seed	1.5	ADF	18.9
Sugar beet pulp	1.75	Ash	7.8
NaCl (Iodized)	0.12	NE_L (Mj/Kg DM)***	0.42
Lime stone	1.03	Calcium	1.15
Dicalcium phosphate	0.75	Phosphorus	0.53
Sodium bicarbonate	0.45		
Rumen protected dry fat*	1.8		
Mineral and vitamin premix**	0.1		
Molasses	3.0		

*Super Sp-202, produced by Allgreen Co. (Malaysia) a special form of a hydrogenated triglyceride which has min. 99% fat as palm oil, acid value, 220 max, melting point, 55°C min.

**Cattle premix produced by Centraly's Co. (France) contains the following elements per Kg. (10000000 IU (Vitamin A), 1000000, IU (Vit. D3), 10000 mg (Vit. E), 100000 mg (magnesium), 50000 mg (manganese), 45000 mg (zinc), 50000 mg (iron), 6000 mg (copper), 800 mg (Iodine), 100 mg (Selenium).

***Calculated according to National research council (2001) NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, NE_L = Net Energy of Lactation

were not contaminated with saliva. The pH was measured immediately using pH meter (Orion research model 201). The whole contents were squeezed through 4 layers of cheesecloth. The samples were acidified to pH 2 with 50% H_2SO_4 and frozen at -20°C for later determination of Volatile Fatty Acids (VFA) concentrations.

Blood sampling: Five cows from each treatment group were randomly selected for blood sampling. Blood samples were collected at 8 and 12 weeks after calving. Blood samples were obtained by vein puncture during or immediately after evening milking. Blood was collected into a 20 ml tube and allowed to clot at ambient temperature. Blood samples were centrifuged at 3000 rpm for 15 min. Only clear non-hemolyzed sera were obtained and kept frozen until further analysis. Samples were analyzed for glucose, cholesterol, triglyceride and blood urea according to Trinder (1969), Zak *et al.* (1954), Sidney and Barnard (1973) and Coulomb and Faverau (1963), respectively. Serum samples were used for the NEFA assay, following the procedures from the NEFA C kit by Wako Chemicals USA, Inc. (ACS-ACOD method), Richmond, V.A. the NEFA assay was performed on one day, with an intra assay-coefficient of variation of 2.63%.

Chemical analysis: Analytical DM contents of TMR, feed ingredients and feces were determined by oven-drying at 105°C for 48 h (AOAC, 1990). Ash contents of TMR, feed ingredients and feces were determined by incineration at 550°C overnight.

Crude protein in TMR and feed ingredient were determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963). NDF and ADF in TMR and feed ingredients were determined according to (AOAC, 1990). Protein and fat concentrations in milk samples were analyzed (AOAC, 1990) by infrared spectrophotometer (System 6000 Milko Scan; Foss Electric). Concentrations of VFA in ruminal fluid were analyzed by calorimetric (Weatherburn, 1967) by GLC (Varian 3700; Varian Specialties Ltd, Brockville, Ontario, Canada).

Statistical analysis: The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 1996) to assess significant differences between group means for the different studied parameters.

RESULTS AND DISCUSSION

Body weight and dry matter intake: There were no differences in body weight of the limited MET cows and RP-MET and/or RP-CHOL supplementation (Table 2). Control group (No. 1) showed a decrease in body weight means by about 4.1, 5.1 and 5.6% until weeks 4, 8 and 12 post-partum respectively, when compared with 1st day post-partum weight. By contrast RP-MET and RP-CHOL treated cows lost only (3.0, 4.0 and 5.1%) and (2.7, 4.3 and 4.3%) less weight respectively, at the same post-partum period when compared with the initial postpartum weight of the same group. Moreover, RP-CHOL and RP-MET treated cows lost 3.5, 4.8 and 4.7% less weight at the same postpartum period when compared with initial postpartum weight at the same group. The difference between limited MET cows and treated groups were not significant ($p \geq 0.05$) indicating

Table 2: Effect of dietary rumen protected methionine and/or choline supplementation on body weight changes (kg/cow) of early lactating cows

Stage of production	Methionine levels	Without choline supplementation	With choline supplementation
No. of observations		15	15
1st day post-partum	Limited MET	603±2.5^{ax}	599±2.5^{ax}
	MET supplement	603±2.5^{ax}	601±2.7^{ax}
4 weeks post-partum	Limited MET	578±2.1^{ax}	583±2.0^{ax}
	MET supplement	585±2.7^{ax}	580±2.4^{ax}
8 weeks post-partum	Limited MET	572±2.0^{ax}	573±1.6^{ax}
	MET supplement	579±2.5^{ax}	572±2.0^{by}
12 weeks post-partum	Limited MET	569±1.5^{ax}	573±1.4^{ax}
	MET supplement	572±2.1^{ax}	573±1.7^{ax}

Values are means±standard error. Mean values with different letters at the same column within supplement (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

that cows fed on diets supplemented with RP-MET and/or RP-CHOL were mobilize less body tissue in the post-partum period. The present data are supported by Davidson *et al.* (2008) who stated that RP-MET., RP-Choline and RP-betaine had no effect on body weight means of dairy cows. Also, Flores *et al.* (2009) reported that RP-MET initial and final body weight of dairy goats were not affected by RP-MET supplementation. On the other hand the data are in contrast with those obtained by Hartwell *et al.* (2000) who indicated that cows fed 12 g of choline per day lost more body weight and lost body weight more rapidly up to 28 day post-partum than cows fed zero or 6 g choline daily.

RP-MET supplementation improved Dry Matter Intake (DMI) by 0.9%, 0.6% during 1st and 2nd month post-partum respectively, when compared with methionine limited cows (Table 3), but this improvement was not significant. While at 12 weeks post-partum, it improved ($p \geq 0.05$) DMI by 0.7%. In addition, the average overall improvement of DMI across the whole experimental period with RP-MET supplementation group was 0.7% when compared with methionine limited cows. These finding are in agreement with those obtained by Davidson *et al.* (2008) who reported that RP-MET supplementation had no effect on average daily DMI of dairy cows.

RP-CHOL supplementation improved DMI (Table 3) by (4.2 and 3.9%), (9.4 and 6.6%) and (10.1 and 6.4%) during 4th, 8th and 12th weeks post-partum when compared with methionine limited and RP-MET + RP-CHOL treated cows respectively. In addition the average overall improvement of DMI across the whole experimental period with RP-CHOL supplementation group was 8.4 and 5.8% when compared with methionine limited and RP-MET + RP-CHOL treated cows respectively. Generally choline supplementation improved DMI more than methionine and this difference may be attributed to the slight sulfur smell of the RP-MET and that explanations were supported by (Benefield *et al.*, 2009) who stated that DMI was decreased when mepron or smartamine rumen protected methionine was fed. These finding are in agreement with those obtained by Zahra *et al.* (2006) who found that there was a conditional effect of supplementing transition dairy cows with RP-CHOL on DMI, such that fat cows that received RP-CHOL ate an average 1.1 Kg/day more from 3 week before calving through 4 weeks post-partum. The results are in contrast with those reported by Erdman and Sharma (1991) which indicated that supplementing early lactation cows with 0, 15, 30 or 45 g/day of RP-CHOL did not affect DMI. Similarly, Piepenbrink and Overton (2003) fed cows 0, 45, 60 or 75 g/day of RP-Choline throughout the transition period and found no effect of RP-CHOL supplementation on DMI. Likewise, Pinotti *et al.* (2003) found no influence of choline supplementation on DMI when transition cows fed 20 g/day of RP-CHOL. It is generally accepted that an increase in DMI is correlated with an increase in milk production.

Production performance: Concerning milk production it was observed that there was a non significant improvement of the RP-MET cows by 2.4 and 1.7% during the 4th and 12th week post-partum respectively when compared with methionine limited group (Table 4), while milk production significantly improved with RP-MET supplementation by 8.9 during 8th week after calving when compared with methionine limited group. Moreover, RP-MET improved milk production across the whole experimental period by 2.5% when compared with methionine limited cows. The results are in agreement with those obtained by Overton *et al.* (1996) who reported that supplemental methionine in rumen protected form, increased the yield of 3.5% Fat Corrected Milk (FCM) when ground shelled corn was the primary fermentable carbohydrate source.

Rumen protected choline supplementation improved milk production by (4.1 and 4.4%), (5.5 and 2.1%) and (7.0 and 2.8%) during 4th, 8th and 12th weeks after calving when compared with methionine limited and RP-CHOL + RP-MET supplemented group respectively. Moreover, RP-CHOL supplementation improved milk production across the whole experimental period by 5.9 and 3.3% when compared with limited methionine cows and

Table 3: Effect of dietary rumen protected methionine and/or choline supplementation on Dry Matter Intake (DMI) of early lactating cows

		Without choline supplementation		With choline supplementation	
Stage of lactation	Methionine level	Kg/day	% from BW	Kg/day	% from BW
Number of observations		28	28	28	28
0-4 weeks post-partum	Limited MET	18.01±0.13^{ay}	3.1	18.76±0.08^{ax}	3.19
	MET supplement	18.17±0.08^{ax}	3.1	18.05±0.07^{bx}	3.12
5-8 weeks post-partum	Limited MET	20.86±0.13^{ay}	3.69	22.82±0.09^{ax}	3.94
	MET supplement	20.99±0.11^{ay}	3.60	21.41±0.12^{bx}	3.68
9-12 weeks post-partum	Limited MET	21.68±0.10^{ay}	3.78	24.06±0.09^{ax}	4.17
	MET supplement	21.83±0.14^{ay}	3.87	22.61±0.07^{ax}	3.94
Average	Limited MET	20.18±0.18^{ay}	3.52	21.88±0.26^{ax}	3.77
	MET supplement	20.33±0.18^{ay}	3.52	20.69±0.22^{bx}	3.58

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

Table 4: Effect of dietary rumen protected methionine and/or choline supplementation on lactation performance (kg/cow) of early lactating cows

Stage of lactation	Methionine levels	Without choline supplementation	With choline supplementation
No. of observations		28	28
0-4 weeks post-partum	Limited MET	29.6±0.16^{ay}	30.8±0.17^{ax}
	MET supplement	30.3±0.14^{ax}	29.5±0.47^{bx}
5-8 weeks post-partum	Limited MET	32.5±0.17^{ay}	34.3±0.16^{ax}
	MET supplement	33.5±0.15^{bx}	33.6±0.14^{bx}
9-12 weeks post-partum	Limited MET	34.3±0.16^{ay}	36.7±0.18^{ax}
	MET supplement	34.9±0.14^{by}	35.7±0.11^{bx}
Average	Limited MET	32.1±0.23^{by}	34.0±0.28^{ax}
	MET supplement	32.9±0.23^{ax}	32.9±0.33^{bx}

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

RP-CHOL + RP-MET supplemented cows respectively. The positive effect of RP-CHOL on milk production are supported by Erdman and Sharma (1991) who reported that RP-CHOL supplementation tended to increase milk yields in early lactation, while significantly improving milk production when supplemented in mid-lactation. Also, Pinotti *et al*. (2003) reported a significant positive effect of RP-CJOL supplementation on milk production of 2.9 Kg/day for the first month of lactation in dairy cows. Similarly, Zahra *et al*. (2006) indicated that RP-CHOL supplementation significantly improved milk production in the first 60 day of lactation by 1.2 kg/day when compared with animals that did not receive RP-CHOL. In contrast, Piepenbrink and Overton (2003) reported no significant increase in milk yields when cows were supplemented with RP-CHOL. In the present study, the increase in milk production appears to be attributed to increased consumption among the RP-CHOL supplemented cows and feeding RP-CHOL may spare methionine catabolism in early lactation due to extensive use of methionine for choline synthesis (Dawson *et al*., 1981; Emmanuel and Kennelly, 1984).

There was a little reduction of milk fat content with RP-MET supplementation by 4.4, 1.7 and 0.0% during 4th, 8th and 12th week after calving when compared with methionine limited cows (Table 5). Moreover, RP-MET reduced milk fat percentage by 2% across the whole experimental period when compared with methionine limited cows. There was no difference in milk fat yield between RP-MET supplemented group and methionine limiting one. On the other hand RP-CHOL and RP-CHOL + RP-MET supplementation reduced ($p \geq 0.05$) milk fat percentage when compared with methionine limited cows, but RP-CHOL increased average milk fat yield by 2.1% when compared with other treatment and this may be attributed to higher milk yield of RP-CHOL supplemented cows. These data are in agreement with those obtained by several authors (Leonardi *et al*., 2003; Noftsger and St-Pierre, 2003; Brodrick *et al*., 2008; Preynat *et al*., 2009) who reported that RP-MET supplementation did not affect milk fat percentage or yield. In contrast Socha *et al*. (2008) reported that duodenal infusion of MET increased milk fat percentage and yield in cows during early lactation, also Misciatteilli *et al*. (2003) determined that early lactation cows fed RP-MET had increased milk fat percentage compared with control cows. Specific mechanisms by which MET supplementation may affect milk fat, including ruminal effects (Patton *et al*., 1968) or postabsorptive effects on lipid metabolism (Emmanuel and Kennelly, 1984) or lipoprotein metabolism remain largely speculative. Nevertheless, results across experiments appear to be quite inconsistent with regard to stage of lactation in terms of whether increased milk fat percentage is accompanied by increased milk fat yield.

Table 5: Effect of dietary rumen protected methionine and/or choline supplementation on milk fat and protein content of early lactating cows

		Without choline supplementation		With choline supplementation	
Stage of lactation	Methionine level	%	Yield (kg/day)	%	Yield (kg/day)
Number of observations		28	28	28	28
Milk fat					
4 weeks post-partum	Limited MET	3.15±0.04^{av}	0.93±0.03^{ax}	2.96±0.05^{ax}	0.91±0.02^{ax}
	MET supplement	3.01±0.04^{bx}	0.91±0.01^{ax}	2.99±0.03^{ax}	0.88±0.01^{bx}
8 weeks post-partum	Limited MET	3.00±0.04^{ax}	0.97±0.01^{ax}	2.92±0.04^{ax}	1.00±0.01^{ax}
	MET supplement	2.95±0.03^{ax}	0.99±0.01^{ax}	2.93±0.04^{bx}	0.99±0.01^{ax}
12 weeks post-partum	Limited MET	2.94±0.04^{ax}	1.01±0.02^{av}	2.90±0.04^{ax}	1.06±0.01^{ax}
	MET supplement	2.94±0.04^{ax}	1.02±0.01^{av}	2.93±0.03^{ax}	1.03±0.01^{by}
Average	Limited MET	3.03	0.97	2.93	0.99
	MET supplement	2.97	0.97	2.95	0.97
Milk protein					
4 weeks post-partum	Limited MET	3.02±0.02^{av}	0.89±0.01^{av}	3.000±0.02^{ax}	0.92±0.01^{av}
	MET supplement	3.05±0.03^{ax}	0.92±0.01^{bx}	3.00±0.04^{ax}	0.88±0.01^{by}
8 weeks post-partum	Limited MET	2.94±0.03^{ax}	0.95±0.01^{av}	3.00±0.04^{ax}	1.03±0.02^{ax}
	MET supplement	3.05±0.04^{ax}	1.02±0.01^{bx}	3.02±0.03^{bx}	1.01±0.01^{bx}
12 weeks post-partum	Limited MET	2.81±0.04^{ax}	0.97±0.02^{av}	2.92±0.07^{ax}	1.08±0.02^{ax}
	MET supplement	2.98±0.06^{ax}	1.03±0.02^{ax}	2.95±0.03^{ax}	1.04±0.01^{bx}
Average	Limited MET	2.92	0.94	2.97	1.01
	MET supplement	3.03	0.99	2.99	0.98

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

There was no significant effect of RP-MET and/or RP-CHOL supplementation on milk protein percentage when compared with unsupplemented cows (Table 5). Moreover, RP-MET increased milk protein percentage by 3.8%, while RP-CHOL and RP-CHOL + RP-MET increased milk protein percentage by 1.7 and 2.4% respectively across the whole experimental period when compared with methionine limited group. Milk protein yield tended to increased with RP-MET and RP-CHOL supplementation by 5.3 and 7.4% across the whole experimental period respectively, when compared with limited methionine cows and this milk protein increment may be due to higher milk production with higher milk protein percentage. This suggests that milk protein synthesis (grams per day) is more responsive to MET supply in higher producing cows than lower producing cows later in lactation (Socha *et al*., 2008; Benefield *et al*., 2009) and the modest response of milk protein percentage to RP-MET + RP-CHOL supplementation may result from the substantially positive MET balance and choline sparing effect of MET (Lobley *et al*., 1996).

Feed efficiency: Energy Corrected Milk (ECM) non significantly improved with cows fed on diet supplemented by 20 g/day of RP-MET by 0.4% during the first four weeks after calving (Table 6) when compared with cows fed on limited methionine diet, but significantly improved at 8th and 12th week postpartum by about 3.0 and 2.5% respectively when compared with control one. Moreover, RP-MET supplementation improved ECM across the whole experimental period by 2.3% when compared with unsupplemented cows.

Rumen protected choline supplementation improved ECM (Table 6) by 0.7, 4.6 and 7.0% at 4th, 8th and 12th week post-partum respectively when compared with cows fed on control diet without any supplement. Moreover, RP-CHOL supplementation improve ECM by about 4.3% across the whole experimental period when compared with the control.

RP-MET supplementation improved milk-to-feed ratio by about 1.2, 2.6 and 1.3% at 4th, 8 th and 12 th week respectively when compared with cows fed on diet without supplementation. Moreover, RP-MET improved milk-to-feed ratio by 1.3% across the whole experimental period when compared with the control. On the other hand, RP-CHOL supplementation tended to slight reduction in milk-to-feed ratio by about 2.5% across the whole experimental period when compared with the control and this may be attributed to higher DMI with RP-CHOL supplementation. The results are in agreement with those obtained by Davidson *et al*. (2008) who stated that ECM yield was higher in cows fed RP-CHOL than cows on all other treatment (methionine limited, RP-MET, betain supplementation) and feed efficiency, reported as kilograms of ECM per kilogram of DMI, was not different as a result of dietary treatment.

Rumen fermentation characteristics: There was no significant difference in ruminal fluid pH (Table 7) as a result of dietary treatment, however, RP-CHOL without or with RP-MET supplementation non significantly reduced rumen pH by 1.6 and 1.6% when compared with limited Met and RP-Met treated cows respectively. Dietary supplementation of unprotected choline has resulted

Table 6: Effect of dietary rumen protected methionine and/or choline supplementation on feed conversion efficiency and Energy Corrected Milk (ECM) of early lactating cows

Items	Methionine levels	Without choline supplementation	With choline supplementation
No. of observations		28	28
Energy Corrected Milk (ECM)*			
0-4 weeks post-partum	Limited MET	28.5±0.17^{ax}	28.7±0.18^{ax}
	MET supplement	28.6±0.17^{ay}	27.6±0.08^{bx}
5-8 weeks post-partum	Limited MET	30.4±0.17^{ay}	31.8±0.20^{ax}
	MET supplement	31.3±0.20^{bx}	31.5±0.18^{bx}
9-12 weeks post-partum	Limited MET	31.5±0.21^{ay}	33.7±0.27^{ax}
	MET supplement	32.34±0.27^{bx}	32.0±0.47^{bx}
Average	Limited MET	30.1	31.4
	MET supplement	30.8	30.4
Milk-to-feed ratio			
0-4 weeks post-partum	Limited MET	1.65±0.01^{ax}	1.64±0.01^{ax}
	MET supplement	1.67±0.01^{bx}	1.63±0.03^{bx}
5-8 weeks post-partum	Limited MET	1.56±0.01^{ay}	1.51±0.01^{ax}
	MET supplement	1.60±0.01^{bx}	1.57±0.01^{bx}
9-12 weeks post-partum	Limited MET	1.58±0.01^{ax}	1.53±0.01^{ax}
	MET supplement	1.60±0.01^{ax}	1.58±0.01^{ax}
Average	Limited MET	1.60±0.01^{ax}	1.56±0.28^{ay}
	MET supplement	1.62±0.01^{ax}	1.59±0.33^{by}

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$). *ECM = {milk (kg/d) x 0.432} + {fat (kg/d) x 12.86} + { protein (kg/d) x 7.65}

Table 7: Effect of dietary rumen protected methionine and/or choline supplementation on rumen fermentation characteristics of early lactating cows

Items	Methionine levels	Without choline supplementation	With choline supplementation
No. of observations		28	28
pH	Limited MET	6.4±0.06	6.3±0.11
	MET supplement	6.4±0.11	6.3±0.10
Total Volatile Fatty Acids (TVA (mm)	Limited MET	92.12±0.90	93.52±0.92
	MET supplement	93.45±0.69	93.03±0.98
Acetate (A) mol/100 ml	Limited MET	62.48±0.59	63.58±1.04
	MET supplement	62.32±0.41	63.97±0.44
Propionate (P) mol/100 ml	Limited MET	20.58±0.36	19.67±0.64
	MET supplement	20.93±0.30	19.58±0.28
Butyrate (mol/100 ml)	Limited MET	16.93±0.57	16.75±0.46
	MET supplement	16.78±0.22	16.45±0.22
Acetate:Propionate ratio (A:P ratio)	Limited MET	3.04	3.23
	MET supplement	2.98	3.27

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

in decreased rumen pH (Sharma and Erdman, 1988), so inadequate protection could result in lowered rumen pH, which did not occur in the current experiment.

RP-MET treated cows recorded a higher ($p \geq 0.05$) total volatile fatty acids (Table 7) by 1.4% when compared with limited Met group, also RP-CHOL treatment group follow the same trend. Moreover, molar proportions (mol/100 ml) and the expected daily production of acetate, propionate and butyrate were not significantly different as a result of dietary treatment. However, RP-CHOL treated cows had non significantly lower molar proportion of propionate and higher acetate. As a result, the ratio of acetate to propionate tended to be higher than RP-MET or limited MET groups. Overall, there were very limited effects on ruminal fermentation characteristics of dairy cows as a result of supplementing RP-MET, RP-CHOL or both RP-MET + RP-CHOL to a Met-limited TMR. Because there were minimal effects on rumen fermentation, these results suggest that RP-MET and Choline products tested in this experiment were adequately protected from ruminal degradation. This result is supported by Atkins *et al.* (1988) who investigated the effects of unprotected choline supplementation on rumen fermentation and reported a higher percentage of acetate in choline supplemented cows than control. Also Davidson (2006) indicated that there were very limited effects on ruminal fermentation in continuous culture as a result of supplementing RP-MET, betaine, or choline to a MET-limited corn silage-based TMR.

Table 8: Effect of dietary rumen protected methionine and/or choline supplementation on some selected blood serum constituents of early lactating cows

Items	Methionine levels	Without choline supplementation	With choline supplementation
No. of observations		28	28
Glucose (mg/dl)	Limited MET	56.6±0.66^{ay}	58.9±0.70^{ax}
	MET supplement	58.1±0.81^{ax}	57.0±0.85^{bx}
Cholesterol (mg/dl)	Limited MET	190.8±2.28^{ay}	208.1±3.32^{ax}
	MET supplement	173.2±2.00^{by}	195.6±2.19^{bx}
Triglyceride (mg/dl)	Limited MET	15.03±0.30^{ay}	14.43±0.70^{ax}
	MET supplement	15.05±0.17^{ax}	14.60±0.85^{ax}
NEFA (mEq/l)	Limited MET	0.57±0.01^{ay}	0.50±0.01^{ax}
	MET supplement	0.55±0.02^{ay}	0.51±0.01^{ax}
Urea N. (mg/dl)	Limited MET	14.71±0.15^{ax}	13.99±0.16^{ax}
	MET supplement	14.35±0.17^{ax}	14.22±0.16^{ax}

Values are means±standard error. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at ($p \leq 0.05$)

Blood serum parameters: There was no significant ($p \geq 0.05$) effect of RP-MET supplementation o serum glucose concentration (Table 8), while RP-CHOL treated cows recorded significantly higher blood serum glucose concentration by 4.1 and 3.3% when compared with limited MET cows and RP-MET + RP-CHOL treated cows respectively. The obtained data are in agreement with Hartwell *et al*. (2000) who reported that RP-CHOL supplementation for dairy cows tended to increase average plasma glucose concentrations. The role of Choline in glucose metabolism is not apparent; however, reducing the severity of lipid accumulation in liver would favor hepatic gluconeogenesis (Cadorniga-Vaino *et al*., 1997) and act to increase blood glucose concentrations in the absences of changes in peripheral glucose utilization.

RP-MET treated cows had significantly lower serum total cholesterol concentration (173.2±2.0 mg/dl) than those fed limited MET diet (190.8±2.28), RP-CHOL treated cows (208.1±3.32 mg/dl) and both RP-CHOL + RP-MET treated cows (195.6±2.19). This is supported by the data obtained by Davidson *et al*. (2008). However, the results disagree with those obtained by Pinotti *et al*. (2003) who reported that supplementing transition dairy cows with 20 g/d of RP-CHOL had no effect on serum cholesterol concentrations. The lower dose of RP-CHOL (12 g/d) in Pinotti *et al*. (2003) may explain the difference, also Zahra *et al*. (2006) reported that supplementation of transition cows with RP-CHOL significantly decreased serum concentrations of cholesterol in the week before calving and the difference may be related to the period of blood sample collection as in this study (the analysis conducted on the sample collected during the 12th week postpartum).

RP-MET had no significant effect o blood serum triglyceride (Table 8) when compared with control (no treatment) cows. This is supported by those obtained by Davidson *et al*. (2008). On the other hand, RP-CHOL treated cows exhibited lower serum triglyceride concentrations when compared with other RP-MET or unsupplemneted cows. Moreover, the current study indicated that RP-MET supplementation in dairy cows diet had no significant effect on blood serum NEFA concentrations but, RP-CHOL without or with RP-MET significantly ($p \leq 0.05$) reduced blood serum NEFA by 12.3 and 7.3% when compared with Met limited and Met treated cows respectively. The effect of choline to decrease blood NEFA of dairy cows may be the results of increased NEFA clearance, decreased adipose tissue breakdown or both (Hartwell *et al*., 2000). The results are in agreement with those obtained by (Pinotti *et al*., 2003) who reported that cows fed 20 g/d of RP-CHOL had decreased circulating concentration of NEFA on the day of parturition compared to control but in contrast with those obtained by (Piepenbrink and Overton, 2003; Janovick Guretzky *et al*., 2006).

Generally at the beginning of the lactation cycle, the plasma level of NEFA originating from the adipose tissue is elevated mainly due to a negative energy balance (Overton and Waldron, 2004). If the uptake of NEFA into the liver exceeds the liver utilization (oxidation and synthesis of ketone bodies) and export of fatty acids from the liver in the form of VLDL, excess triglycerides are stored in heptocytes resulted fatty liver (Bobe *et al*., 2004) and may lead to an impaired capacity of the liver for gluconeogenesis and would result in a reduced performance. In the present study RP-CHOL reduced both NEFA and triglycerides in the blood serum which may be reflected on the liver health conditions and the efficacy of choline was more clear than methionine supplementation as a methyl group donor and most of the potential application of choline in the nutrition of dairy cows has focused on its role in lipid metabolism.

The concentrations of serum urea N were not different in response to either treatment or parity, suggesting that overall N utilization was similar across parties and treatments. This result is supported by those obtained by (Zahra *et al*., 2006; Davidson *et al*., 2008).

Conclusion: Dietary RP-CHOL (30 g/day) to early lactating dairy cows that received a MET limited diet improved DMI, milk yield and increased milk protein yield. In this study, supplementing RP-MET (15 g/day) or both RP-MET + RP-CHOL were not beneficial as RP-CHOL supplementation alone. Data possibly indicates that supplementary RP-CHOL exonerates MET metabolism due to interchangeable methyl groups. Moreover supplementary RP-CHOL alone to MET limited diet showed higher positive effects on health and production which is mainly attributed to lipid metabolism and due to supply of additional methyl groups and we conclude that if a deficiency of methyl groups in dairy cows is likely to occur, RP-CHOL appears to be a good choice for supplementation. In conclusion, further investigations are required for a better understanding of the interrelationship between MET and CHOL metabolism and for a quantification of the demand for methyl groups of dairy cows.

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