Effect of Rumen-protected Choline Addition on Milk Performance and Blood Metabolic Parameters in Transition Dairy Cows

Guozhong Xu, Jun'An Ye, Jianxin Liu* and Yueying Yu[1]
Institute of Dairy Science, Zhejiang University, Hangzhou 310029, P. R. China

ABSTRACT : This work was conducted to study the effect of rumen-protected choline (RPC) addition on milk performance and blood metabolic parameters in transition dairy cows. In Experiment 1, fourteen Chinese Holstein dairy cows were supplemented with 0 or 20 g/d of RPC from 7 d before expected calving to 21 d *post partum*. Feeding of RPC tended to increase milk yield and milk protein percentage, while milk fat and lactose percentage were not changed. Plasma concentrations of glucose tended to increase as cows consumed RPC, while plasma concentrations of triglycerides, very low density lipoproteins, cholesterol and nonesterified fatty acids were not significantly different between the two groups. In Experiment 2, thirty-six Chinese Holstein dairy cows were supplemented with 0, 30, 60 or 90 g/d RPC from 15 d before expected calving to 15 d *post partum*. Feeding of RPC tended to increase yield of milk and 4% fat-corrected milk for all the lactating cows, and milk composition was similar among the four groups. Plasma concentrations of glucose were remained at a higher level in 30 or 60 g/d RPC-supplemented groups, and nonesterified fatty acids were decreased in the 30 g/d group. Concentrations of triglycerides tended to reduce in 30 and 90 g/d RPC-supplemented animals, and cholesterol was reduced in 0 or 30 g/d group. These results suggest that RPC addition tended to increase milk yield and improve blood metabolic parameters during transition dairy cows, and feeding 30 g/d of RPC may be the optimal. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 390-395)***

Key Words : Chinese Holstein Dairy Cows, Rumen-Protected Choline, Milk Performance, Blood Metabolic Parameters

INTRODUCTION

Dairy cows experience a dramatic physiological and metabolic adaptation during the transition period accompanied by decreased dry matter intake, while the nutritional requirements for maintenance, pregnancy and milk production increase rapidly (Moe and Tyrrell, 1972; Bell, 1995), resulting in a state of negative energy balance. Therefore, dairy cows must mobilize more body fat to meet their nutritional requirements and may produce higher concentrations of non-esterified fatty acids (NEFA) in the blood plasma during the transition period (Rukkwamsuk et al., 1999). Although the NEFA can be used for milk production and exported to blood as very low density lipoproteins (VLDL) from liver, the excess uptake of NEFA by the liver may cause fatty livers with accumulation of triglycerides. The negative energy balance may also cause other metabolic disorders such as ketosis (Ballard et al., 2001).

Choline is a required nutrient for many animal species. It acts as a methyl donor and plays an important role in phospholipid formation for lipoprotein metabolism. Choline deficiency may rapidly induce fatty liver in rats (Dutta-Roy, 1988). If choline is insufficient in the periparturient dairy cow, the accumulation of triglycerides may increase. Dietary choline can be quickly degraded in the rumen, thus rumen-protected forms may be a practical means of choline addition (Atkins et al., 1989). When 20 g/d of a rumen-protected choline (RPC) was fed to dairy cows starting 14 d before expected calving date through 30 DIM, more milk was produced (Pinotti et al., 2001). Piepenbrink et al. (2003) observed that adding RPC at 45 and 75 g/d tended to increase the concentration of glycogen, and the rate of VLDL synthesis in and secretion of esterified lipid products from liver, whereas the milk yield and body condition were not affected. In contrast, Hartwell et al. (2000) reported that RPC-fed cows lost more weight and tended to be in a worse body condition, compared to controls without the RPC. Meanwhile, Pinotti et al. (2004) observed that choline as a component of lipoprotein could increase plasma folate and vitamin E status in periparturient dairy cows. It has been observed that vitamin E has a positive effect on the immune system, and vitamin E supplementation may reduce oxidative stress in periparturient cows (Chatterjee et al., 2003) and buffalo heifers (Kahlon et al., 2004; Sharma et al., 2005).

There is little information on RPC addition in Chinese dairy cows. The objective of this study was to investigate the effects of RPC addition on milk performance and blood metabolic parameters in transition dairy cows.

MATERIALS AND METHODS

Animals and sampling procedures

Experiment 1 : Fourteen multiparous Chinese Holstein cows weighing on average 550±10 kg (entering second or

* Corresponding Author: Jian-Xin Liu. Tel: +86-571-86971097, Fax: +86-571-86971647, E-mail: liujx@zju.edu.cn
[1] Hangzhou Jinjiang Dairy Farm, Hangzhou 310016, P. R. China.
Received March 10, 2005; Accepted August 19, 2005

Table 1. Ingredients and chemical compositions of the diets used in Experiments 1 and 2

Composition	Experiment 1		Experiment 2	
	Pre-partum	*Post-partum*	*Pre-partum*	*Post-partum*
Ingredients (g/kg)				
Concentration mixture[1]	422	512	447	502
Chinese wildrye	324	53	365	42
Maize silage	254	221	188	242
Alfalfa haylage	-	214	-	214
Chemical composition (g/kg DM)				
Crude protein	113	125	10.9	12.0
Neutral detergent fibre	331	336	33.8	33.4
Acid detergent fibre	153	157	15.6	15.4
Ca	7.2	0.93	0.49	0.71
P	3.5	0.48	0.27	0.39

[1] Ingredients (g/kg): maize 650, wheat bran 160, soybean cake 45, cottonseed 125, mineral and vitamin mixture 10 and sodium chloride 10.

Table 2. Production responses of early lactation cows to addition of rumen-protected choline in Experiment 1

	Rumen-protected choline addition (g/d)		SEM
	0	20	
DMI[1] (kg/d)	14.2	14.8	0.5
Milk (kg/d)	24.4	26.4	1.3
4% FCM[2] (kg/d)	30.7	33.3	1.7
Milk fat (g/kg)	57.5	57.7	4.3
Milk protein (g/kg)	31.4	32.5	2.6
Milk lactose (g/kg)	46.4	45.7	1.8

[1] DMI = Dry matter intake.
[2] FCM = 0.4×(kilograms of milk yield)+15×(kilograms of milk fat).

greater lactation) were selected during 14 d *pre-partum*. All cows were fed the same diet *pre-partum* (Table 1). On d 7 before expected calving, cows were randomly assigned to two groups (0 or 20 g/d RPC) according to the expected calving date, parity and previous milk production. The RPC contained 37.5% choline chloride, which was approximately 70% rumen-protected. The level of RPC addition was set according to previous studies by others (Sharma et al., 1989; Pinotti et al., 2001). All cows were fed three times daily and housed in individual tie stalls fitted with rubber mats and bedded with wood shaving. Feed refusals were recorded once daily. Cows had free access to water and were provided with 2 h of exercise daily.

Cows were milked three times daily at equal intervals, and milk yield was measured once each week. Milk was sampled from each milking during a 24 h period, all the samples were mixed and analyzed for fat, protein and lactose content by infrared analysis (Milk-o-Scan, Foss Electric, Denmark).

Blood samples were obtained by venipuncture of the coccygeal vein of each cow using heparinized vacutainer tubes after morning feeding at weekly intervals from 7 d *pre-partum* to 21 d *post-partum*. Plasma samples were collected after centrifugation of the blood at 3,000 g for 15 min. Plasma was stored at -20°C until analysis for NEFA (Sheldon et al., 1981), glucose, glutamate-oxaloacetic transaminase, triglycerides and cholesterol (Kits, Ningbo Cicheng Bioengineering Institute, China).

Feedstuffs were sampled weekly and stored at -20°C for later analysis. Composited feed samples were dried at 65°C for 48 h and passed through a 1-mm screen. The feeds were analyzed for dry matter (DM), crude protein (CP) and crude fiber according to the methods of Association of Official Analytical Chemists (AOAC, 1995), and for neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the methods of Van Soest et al. (1991).

Experiment 2 : Thirty-six cows (24 multiparous and 12 primiparous) were equally divided into four groups by date of calving and parity. On the basis of results in Experiment 1, four doses of RPC (0, 30, 60 and 90 g/d) were added to determine the optimal level. Cows were milked three times daily at equal intervals, and milk yield was measured once every five days. Blood samples were obtained by venipuncture of the coccygeal vein of each cow using heparinized vacutainer tubes after morning feeding at weekly intervals from 7 d *pre-partum* to 15 d *post-partum.*

The management of animals and measurements of milk composition, blood metabolic parameters and feed samples were the same as described in Experiment 1.

Statistical analysis

The data were analyzed by ANOVA, using the general linear model (PROC GLM) and Duncan procedures (SAS, 1999) for the model: Y = covariate+block+treatment+ residual error. Analyses were made for milk performance and blood metabolic parameters for each weekly point and the whole mean of the experiment. Significance was declared at $p<0.05$.

RESULTS

Milk yield and composition

In Experiment 1, *post-partum* DM intake by cows was not affected by RPC addition (Table 2). The RPC-supplemented cows tended to produce more milk than those

Table 3. Effects of rumen-protected choline addition on dry matter intake (DMI), and milk yield and composition in Experiment 2

	Rumen-protected choline addition (g/d)				SEM
	0	30	60	90	
For all cows					
DMI (kg/d)	14.9	15.2	15.6	15.9	0.6
Milk (kg/d)	25.4	29.5	27.1	24.9	2.5
4% FCM[1] (kg/d)	27.7	34.0	29.0	29.2	4.2
Milk fat (g/kg)	47.4	49.2	47.0	50.2	3.5
Milk protein (g/kg)	33.7	31.5	32.3	34.3	1.4
Milk lactose (g/kg)	46.1	46.3	47.0	45.0	1.7
Total solids (g/kg)	134.2	133.4	133.8	139.9	4.7
For multiparous cows					
Milk (kg/d)	26.1 [ab]	30.0 [a]	28.3 [ab]	25.8	1.4
4% FCM[2] (kg/d)	28.4[ab]	35.6[a]	31.5[ab]	27.4[b]	2.9
Milk fat (g/kg)	46.9	49.5	47.1	48.5	3.1
Milk protein 9g/kg)	33.8	31.5	32.6	34.8	1.3
Milk lactose (g/kg)	46.9	46.4	47.6	44.7	1.6
Total solids (g/kg)	134.6	136.4	134.2	133.6	4.8

[1] FCM = 0.4×(kilograms of milk yield)+15×(kilograms of milk fat).
[a, b] Means with different superscripts within a row differ p<0.05.

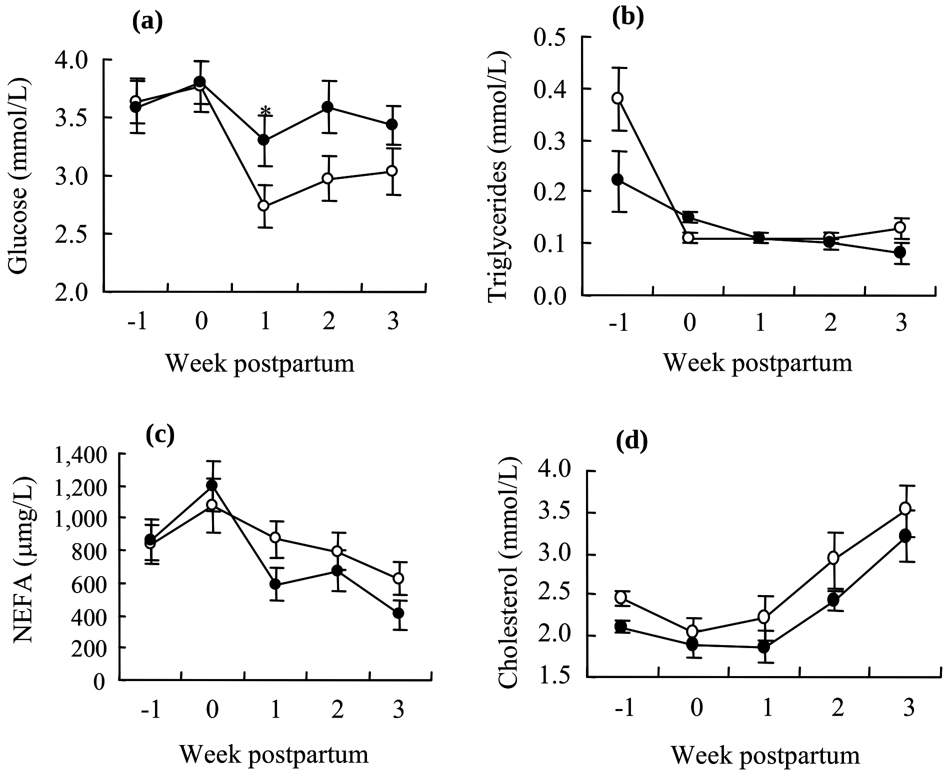

Figure 1. Concentrations of glucose (a), triglycerides (b), NEFA (c) and cholesterol (d) in blood plasma from cows fed 0 (○) or 20 g/d (●) of rumen-protected choline. Differences at individual time points are indicated by * (p<0.05). NEFA = non-esterified fatty acids. Bars indicate standard error. Where not visible, bars fall within symbol.

in the control group (26.4 vs. 24.4 kg/d), though the difference was not statistically significant. Milk yield of all cows increased with lactation, and the milk yield was higher than the control group throughout the experiment, but it was not significantly different. There were no significant differences in percentages of milk fat and lactose. Feeding of RPC resulted in a slight increase in milk protein (3.25 vs. 3.14%) over the control (p>0.05).

In Experiment 2, the *post-partum* DM intake by cows was similar (Table 3). There was a trend to increase milk and FCM yield for cows fed 30 g/d of RPC (29.5 vs. 34.0 kg/d), and there was little differences in these parameters among the groups (p>0.05). As lactation progressed, each group produced more milk after calving, the milk yield was

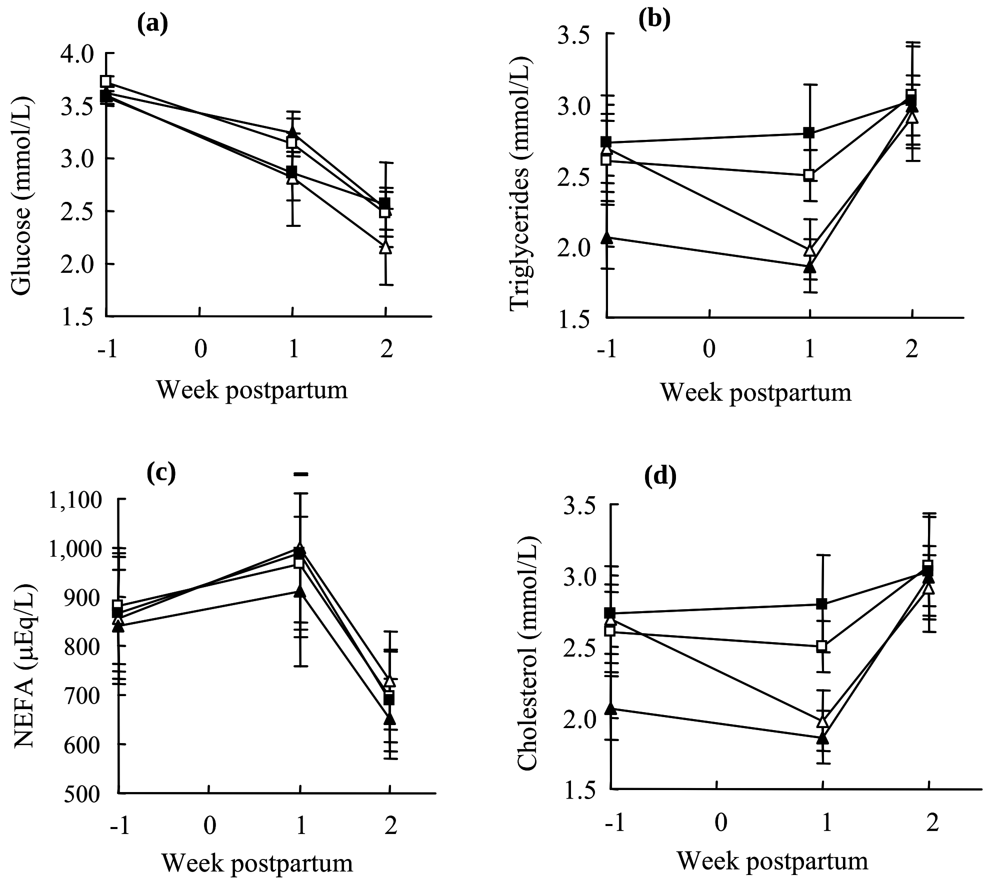

Figure 2. Concentrations of glucose (a), triglycerides (b), NEFA (c) and cholesterol (d) in blood plasma from cows fed 0 (Δ), 30 (▲), 60 (□), or 90 g/d (■) of rumen-protected choline. NEFA = non-esterified fatty acids. Bars indicate standard error. Where not visible, bars fall within symbol.

higher in 30 g/d groups during the whole experiment and no difference was detected among the four groups at each sampling time. Milk fat percentage for cows fed 90 g/d or 30 g/d RPC tended to be higher and milk protein percentage tended to be lower in the 30 g/d RPC group, but there were no significant differences associated with RPC addition ($p>0.05$, Table 3). The percentages of milk lactose and total solids were similar among the four groups. When the data for multiparous cows were analyzed, the differences between the treatments became more pronounced (Table 3). The cows given 30 g/d of RPC produced significantly more milk ($p<0.05$) than those supplemented with 90 g/d RPC ($p<0.05$).

Blood metabolic parameters

Plasma concentration of glucose increased in RPC groups and plasma concentrations of cholesterol, triglycerides and NEFA for RPC-supplemented cows tended to be lower than the control in Experiment 1 ($p>0.05$, Figure 1). Plasma concentration of glucose increased as parturition approached and reached a maximum at the calving point and decreased to a minimum in week 1 *post-partum* ($p<0.05$), then increased as lactation progressed (Figure 1a). Plasma concentrations of triglycerides and NEFA were not significantly different between the two groups, and had the same tendency to decrease *post-partum*, but cholesterol concentration tended to increase at week 1 *post-partum* (Figure 1b, c and d).

In Experiment 2, plasma concentrations of glucose tended to increase as cows consumed RPC ($p>0.05$, Figure 2). Plasma concentrations of cholesterol, triglycerides and NEFA for RPC-supplemented cows tended to be lower than the control ($p>0.05$); when higher levels of RPC were added, the cholesterol concentration tended to be higher. Plasma concentrations of glucose, cholesterol, triglycerides and NEFA had the same trend after calving in all cows (Figure 2), and no difference was detected ($p>0.05$), but in cows fed 30 g/d RPC glucose concentration tended to be higher and triglycerides, NEFA and cholesterol concentration tended to be lower.

DISCUSSION

Some work has been conducted on dietary RPC addition in dairy cows in Western countries, but the results were inconsistent (Dicostanzo and Spain, 1995; Hartwell et al., 2000; Piepenbrink et al., 2003). There were no previous reports on RPC addition in Chinese dairy cows.

In both experiments, RPC addition did not affect DM intake (Tables 2 and 3). This result was in agreement with the conclusion of Piepenbrink et al. (2003), who observed that DM intake was similar for cows supplemented.with 45-75 g/d RPC Other researchers also observed that *post-partum* DM intake was not affected by RPC addition (Hartwell et al., 2000; Piepenbrink et al., 2003), and even in mid-lactation or in finishing beef steers DM intake was not affected (Erdman and Sharma, 1991; Bryant et al., 1999), suggesting that DM intake was not sensitive to supply of RPC during the periparturient period.

Milk yield tended to be higher in the cows receiving a lower level of the RPC (Tables 2 and 3), but did not increase with increasing level of RPC. The results were more pronounced when the data for the multiparous cows were analyzed separately (Table 3). These observations were consistent with those of Pinotti et al. (2001), who reported that cows fed 20 g/d of RPC produced more milk. Feeding of RPC had little or no effect on milk composition, which is affected by many factors such as breed, forage: concentration ratio, etc. In our experiments, we started 7-14 days before expected calving, which may affect the function of the RPC. Some researchers added the RPC from 14-28 days before the expected calving (Pinotti et al., 2001; Hartwell et al., 2000; Piepenbrink et al., 2003).

Plasma NEFA concentrations increase prior to and at parturition, and the rate of triglycerides synthesis is proportional to plasma NEFA concentration (Grummer, 1993). In the present study feeding of RPC tended to increase the plasma concentrations of glucose and decrease the triglycerides or NEFA at the lower level (20-30 g/d) of RPC addition, which would reduce the risks of metabolic disorders in transitional dairy cows. Piepenbrink et al. (2003) observed that RPC-supplemented cows had increased liver concentrations of glycogen and plasma concentrations of glucose, while plasma concentrations of NEFA or triglycerides were not altered. Hartwell et al. (2000) also observed that addition of RPC did not affect the plasma concentrations of NEFA in lactating cows.

In summary, feeding of 30 g/d of RPC may be the optimal, and more RPC addition did not increase milk yield and improve blood metabolic parameters during transition period for Chinese Holstein dairy cows.

ACKNOWLEDGMENTS

We thank Mr. Zemin He and the staff of the dairy farm for their assistance in the experiment. This research was financially supported in part by Ministry of Science and Technology of China and Ministry of Agriculture, China.

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