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Effects of Biotin and Rumen-protected Choline Supplementation on Milk Production, Milk Composition, Live Weight Change and Blood Parameters in Lactating Dairy Cows

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Effects of Biotin and Rumen-protected Choline Supplementation on Milk Production, Milk Composition, Live Weight Change and Blood Parameters in Lactating Dairy Cows

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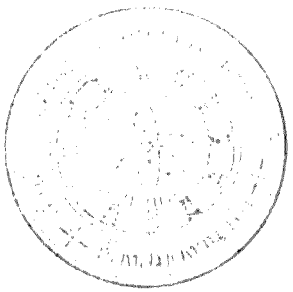

Abstract: The objective of this study was to determine the effects of biotin and rumen-protected choline supplementation on milk production, milk composition, live weight change, milk choline and blood parameters in crossbred Holstein Friesian dairy cows. Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 48 ± 13 days in milk, 15.5 ± 0.8 kg of milk and 380 ± 19 kg body weight, were blocked by milking days first and then stratified random balanced for milk yield and body weight into three groups of 8 cows. The first group (control) received approximately 6 kg of 21% CP concentrate. The second group was fed the same basal diet as the control group and supplemented with 20 mg/d of biotin (BASF (Thai) Co., Ltd.) filled in a capsule and 20 g RPC/d top-dressed (Reashure®, Balchem Co., Ltd.) and the third group was fed the same basal diet as the control group and supplemented with 20 mg/d of biotin filled in a capsule and 40 g RPC/d top-dressed. All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*; 55 d cutting age), 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 first 2 weeks as the adjustment period, followed by 8 weeks of measurement period Feed offered and left after eating of individual cow were collected on 2 consecutive days weekly and at the end of the experiment feed samples were pooled to make representative samples for proximate and detergent analyses. 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 samples were taken on day 56 of the experiment and subjected to milk choline. 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, milk component yields and milk choline were increased by biotin and rumen-protected choline supplementation. It is recommended in the present study that the addition of 20 mg/d biotin and 20 g/d rumen-protected choline could be beneficial to lactating dairy cows in early lactation.

Key Words : Biotin, rumen-protected choline milk production, milk composition, blood parameters, dairy cows.

INTRODUCTION

Biotin, a water soluble, B-vitamin, is essential for the growth of all major rumen bacteria and is also essential for the dairy cow herself. Biotin is a cofactor with enzymes involved in pathways for amino acid metabolism, cellular respiration, and both glucose and fatty acid synthesis. Biotin is required for the rumen fermentation of dietary carbohydrate to propionic acid and for the conversion of propionic acid to glucose in the liver. Biotin is also required in hoof horn formation for the production of structural proteins (keratin) and for the production of intracellular cement that bonds together hoof horn cells to form a semi-waterproof barrier to the environment. Both of these factors affect the integrity of the hoof horn, and ultimately the hoof health of dairy cows.

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; Brüsemeister and Südekum, 2006). Dietary choline is degraded rapidly by the rumen microorganisms (Neill, 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.

Increases in milk yield due to the addition of biotin (Zimmerly and Weiss, 2001; Majee *et al.*, 2003; Bergsten *et al.*, 2003) and RPC (Erdman and Sharma, 1991; Piepenbrink and Overton, 2003; Baldi and Pinotti, 2006; Zahra *et al.*, 2006; Lima *et al.*, 2007; Davidson *et al.*, 2008) were previously observed. Recent research work, Suksombat *et al.* (2011a) fed lactating dairy cows 20

and 40 g RPC/d found significant increases in milk yield and 3.5% FCM due to RPC supplementation while Suksombat *et al.* (2011b) supplemented dairy cows with 20 and 40 mg biotin/d and found no significant differences in milk yield and milk composition. In addition, when 40 mg of biotin was compared to 20 mg there was no additional response (Majee *et al.* 2003). The present study therefore designed to determine the effects of biotin (20 mg/d) and RPC (20 and 40 g/d) on performances of lactating dairy cows.

MATERIALS AND METHODS

Animals and Treatments

Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 48 ± 13 days in milk, 15.5 ± 0.8 kg of milk and 380 ± 19 kg body weight, were blocked by milking days first and then stratified random balanced for milk yield and body weight into three groups of 8 cows. The first group (control) received approximately 6 kg of 21% CP concentrate. The second group was fed the same basal diet as the control group and supplemented with 20 mg/d of biotin (BASF (Thai) Co., Ltd.) filled in a capsule and 20 g RPC/d top-dressed (Reashure®, Balchem Co., Ltd.) and the third group was fed the same basal diet as the control group and supplemented with 20 mg/d of biotin filled in a capsule and 40 g RPC/d top-dressed. Reashure® contains 25% choline in a chemical form of choline-chloride; hence Reashure® fed at 80 and 160 g/d to provide 20 and 40 g/d of choline respectively. All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*; 55 d cutting age), 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 first 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 two consecutive days of each period and samples were taken and dried at 60°C for 48 hour. 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 two consecutive days weekly and stored at 4°C with a preservative (bronopol tablet; D&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 50, 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 to 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, unit definition: 1 unit liberates 1.0 mmol choline from L-a-phosphatidyl choline/h 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, unit definition: 1 unit forms 1.0 mmol H2O2 with oxidation of 1mmol choline to betaine aldehyde/min 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, unit definition: 1 unit forms 1.0 mg purpurogallin from pyrogallol in 20 s 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 523mg of choline bitartarate (Sigma C-2654;

Sigma-Aldrich) in 100 ml of water, which was equal to 2500mg/ml choline hydroxide solution. The five-point standard curve (50, 100, 150, 200 and 250 mg/ml 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 of the experimental period. 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, 1996). Differences between treatment means were statistically compared using Least Significant Differences (Steel and Torrie, 1980).

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 presented 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 d cutting age) and, consequently, resulting in low DM and CP degradability (42.6 and 50.4% 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 biotin plus RPC-supplemented cows is in agreement with several earlier studies (Erdman and Sharma, 1991; Hartwell *et al.*, 2000; Zimmerly and Weiss, 2001; Margerison *et al.*, 2003; Piepenbrink and Overton, 2003; Pinotti *et al.*, 2003; Rosendo *et al.*, 2004; Zahra *et al.*, 2006). However, only one trial, Majee *et al.* (2003) found increases in DMI (0.7 – 1.3 kg/d) when 20 mg/d of biotin was supplemented.

Dry matter (DM), crude protein (CP) and net energy for lactation (NE_{LP}) intakes of the experimental cows were similar (P>0.05) (Table 2). Similar results were previously reported when cows were supplemented with 20 - 30 mg/d of biotin (Zimmerly and Weiss, 2001; Margerison *et al.*, 2003; Rosendo *et al.*, 2004). However, Majee *et al.* (2003) found increases in DMI (0.7 – 1.3 kg/d) when 20 mg/d of biotin was supplemented. The main differences between the three studies and the latter were: different duration of the trials, different forage program with corn or grass silage vs alfalfa silage. How these factors may be related to the different intake response to biotin supplementation between the trials is unclear. Supplementing transition cows with rumen-protected choline before calving did not influence DM intake prepartum, but when supplemented after calving, it tended to increase postpartum intake (Lima *et al.*, 2007). Feed intake responses to supplemental rumen-protected choline have been variable and some studies have reported no effects (Erdman and Sharma, 1991; Hartwell *et al.*, 2000; Piepenbrink and Overton, 2003; Zahra *et al.*, 2006), whereas others observed an increase in DM intake (Oelrichs *et al.*, 2004; Chung *et al.*, 2005). It is unknown the mechanism by which choline might influence DM intake, but it is plausible to speculate an indirect effect mediated by improved postparturient health. Differences in response to rumen-protected choline might also be related to the quality of the product used and method of protection against rumen degradation as differences in the degree of rumen-protection have been shown for different products (Kung *et al.*, 2003).

Recent researches, Suksombat *et al.* (2011a) found no significant differences in milk yield and milk composition in response to biotin supplementation, however, Suksombat *et al.* (2011b) found increases in milk yield and milk component yields due to rumen-protected choline supplementation. Increasing the intestinal supply of choline has usually improved milk production in lactating dairy cows approximately 7 per cent over controls (Baldi and Pinotti, 2006), and this

improvement seemed independent on the dose of rumen-protected choline supplemented to cows, which ranged from 6 to 60 g/d. Erdman and Sharma (1991) conducted 2 experiments to evaluate the effect of different levels of rumen-protected choline and interaction between choline and dietary CP level in cows past 5 wk of lactation. In experiment 1, milk yield tended to increase with feeding of rumen-protected choline, and intake of choline chloride ranged from 17 to 50 g/d. In experiment 2, increasing intake of rumen-protected choline from 0 to 55 g/d of choline chloride resulted in a linear increase in milk yield despite dietary protein content (Erdman and Sharma, 1991). Generally, yields of milk and 3.5 per cent fat-corrected milk in response to rumen-protected choline have either been unaltered (Hartwell *et al.*, 2000; Janovick Guretzky *et al.*, 2006; Davidson *et al.*, 2008) or increased (Erdman and Sharma, 1991; Piepenbrink and Overton, 2003; Zahra *et al.*, 2006; Lima *et al.*, 2007; Davidson *et al.*, 2008). In the present study, it seems likely that increases in milk yield and milk component yields depend solely on rumen-protected choline supplementation since previous research found no significant difference in milk yield due to biotin supplementation (Suksombat *et al.* 2011a) while milk yield was increased by rumen-protected choline supplementation (Suksombat *et al.* 2011b).

Milk component yields were increased by biotin and rumen-protected choline supplementation in the present study. Increased yields of milk components from feeding rumen-protected choline have generally been the result of increased milk yield, with some effects on milk fat content, but little or no effect on milk protein content. Because choline is used for phospholipid synthesis, it has been suggested that supplementation with choline may facilitate lipid absorption and transport, thereby favoring milk fat synthesis (Erdman *et al.*, 1984); however, more detailed work on blood lipids revealed that supplementation with rumen-protected choline did not alter the concentrations of different lipid fractions in plasma of lactating Holstein cows (Janovick Guretzky *et al.*, 2006). In early lactation, plasma nonesterified fatty acids supplies the majority of fatty acids secreted by the mammary gland in dairy cows, and this can increase if weight loss postpartum is exacerbated. Because rumen-protected choline might reduce fat mobilization based on plasma nonesterified fatty acid concentrations (Pinotti *et al.*, 2002; Cooke *et al.*, 2007), it is possible that potential effects of choline on intestinal lipid absorption, hepatic triacylglycerol secretion, and subsequent transport to the mammary gland might be masked by the reduced availability of nonesterified fatty acids for uptake by the mammary cells.

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 authors 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 first 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 (4.31 vs 4.42 and 4.46 mmol/l, in control, 20 mg biotin/ 20 g RPC animals and 20 mg biotin/ 40 g RPC animals), β-hydroxybutyrate (0.54 vs. 0.52 and 0.53 mmol/l), cholesterol (3.82 vs. 4.06 and 4.11 mmol/l), NEFA (0.59 vs. 0.53 and 0.54 mmol/l) or the NEFA/cholesterol ratio (0.15 vs. 0.13 and 0.13) (Table 4).

CONCLUSION

Feeding biotin and RPC did not alter intakes, milk composition, live weight change and blood parameters, however, milk yield, 3.5% fat-corrected-milk yield, milk component yields 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/d of RPC could be added to lactating dairy cow's diet for better beneficial response

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

% Dry matter	Concentrate	Grass silage
Dry matter	94.32	28.97
Crude protein	21.37	5.43
Crude fat	4.11	1.59
Ash	8.94	9.09
Crude fiber	12.35	30.29
Non fiber carbohydrate	33.89	15.69
Neutral detergent fiber	38.69	69.47
Acid detergent fiber	16.38	53.32
Acid detergent lignin	4.12	4.60
Neutral detergent insoluble nitrogen	1.12	0.20
Acid detergent insoluble nitrogen	0.41	0.15
TDN_{1X} (%)1	68.93	53.89
DE_P (Mcal/kg)2	2.98	2.41
ME_P (Mcal/kg)3	2.56	1.97
NE_{LP} (Mcal/kg)4	1.62	1.19
Effective degradability of dry matter (*dg*DM)	64.2	42.6
Effective degradability of crude protein (*dg*CP)	67.8	50.4

TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 25.25) + tdNDF – 7)
DE_{1X} (Mcal/kg) = ((tdNFC/100) x 4.2) + ((tdNDF/100) x 4.2) x ((tdCP/100) x 5.6) + ((FA/100) x 9.4) – 0.3
DE_P (Mcal/kg) = (((TDN$_{1X}$ –((0.18 x TDN$_{1X}$) – 10.3)) x Intake)/TDN$_{1X}$) x DE_{1X}
ME_P (Mcal/kg) = (1.01 x (DE_P) – 0.45) + (0.0046 x (EE-3))
NE_{LP} (Mcal/kg) = (0.703 x ME_P) – 0.19 , (EE > 3%)
NE_{LP} (Mcal/kg) = (0.703 x ME_P) -0.19) + ((0.097 x ME_P)/97) x (EE – 30), (EE > 3%)

Table 2 Effects of biotin and rumen-protected choline supplementation on DM, CP and NE_{LP} intakes of dairy cows.

Intake	Control	20 mg biotin + 20 g RPC/d	20 mg biotin + 40 g RPC /d	SEM	P-value
DM (kg)					
Concentrate	7.55	7.55	7.55		
Grass silage	6.24	6.43	6.14	0.23	0.54
Total	13.79	13.98	13.68	0.25	0.52
CP (g/d)					
Concentrate	1613	1613	1613		
Grass silage	339	349	333	15.28	0.56
Total	1952	1962	1946	15.58	0.53
NE_{LP} (Mcal/d)					
Concentrate	12.22	12.22	12.22		
Grass silage	7.43	7.65	7.30	0.32	0.56
Total	19.65	19.87	19.52	0.30	0.54

SEM = standard error of the mean; RPC = rumen-protected choline; NE_{LP} = net energy for lactation at production level

Table 3 Effects of biotin and rumen-protected choline supplementation on milk yield, milk composition, final liveweight and live weight change

Yields	Control	20 mg biotin + 20 g RPC/d	20 mg biotin + 40 g RPC /d	SEM	P-value
Milk yield (kg/d)	15.3[b]	16.2[ab]	16.6[a]	0.29	0.047
3.5% FCM (kg/d)	16.5[b]	17.9[a]	18.4[a]	0.31	0.036
% Fat	3.98	4.13	4.15	0.11	0.446
% Protein	2.99	3.02	3.06	0.09	0.867
% Lactose	4.83	4.87	4.91	0.06	0.892
% Solid-not-fat	8.52	8.59	8.67	0.13	0.947
% Total solid	12.50	12.72	12.82	0.20	0.707
Fat yield (g/d)	609[b]	669[ab]	689[a]	18.9	0.042
Protein yield (g/d)	457[b]	489[ab]	508[a]	12.4	0.045
Lactose yield (g/d)	739[b]	789[ab]	815[a]	16.2	0.042
Solid-not-fat yield (g/d)	1304[b]	1392[ab]	1439[a]	32.1	0.043
Total solid yield (g/d)	1913[b]	2061[ab]	2128[a]	34.5	0.049
Initial live weight (kg)	378	382	381	9.64	0.827
Final live weight (kg)	398	404	406	9.95	0.730
Live weight change (g/d)	+357	+393	+438	116.3	0.958

SEM = standard error of the mean; FCM = fat-corrected-milk; RPC = rumen-protected choline

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

	Control	20 mg biotin + 20 g RPC/d	20 mg biotin + 40 g RPC /d	SEM	P-value
Milk choline (mg/kg)	97.5[b]	136.4[a]	142.7[a]	5.70	0.034
Plasma glucose (mmol/L)	4.31	4.42	4.46	0.07	0.762
Plasma BHBA (mmol/L)	0.54	0.52	0.53	0.04	0.915
Plasma NEFA (mmol/L)	0.59	0.53	0.54	0.04	0.891
Plasma cholesterol (mmol/L)	3.82	4.06	4.11	0.11	0.776
NEFA/Cholesterol	0.15	0.13	0.13	0.02	0.824

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 ($P < 0.05$).