Journal of Animal and Veterinary Advances 10 (16): 2186-2192, 2011
ISSN: 1680-5593

Effects of Biotin Supplementation on Milk Production, Milk Composition, Milk Fatty Acids, Ruminal pH, Ammonia Nitrogen and Volatile Fatty Acids in Lactating Dairy Cows

W. Suksombat, P. Lounglawan and P. Paengsai
School of Animal Production Technology,
Institute of Agricultural Technology, Suranaree University of Technology,
University Avenue, Amphur Muang, 30000 Nakhon Ratchasima, Thailand

Abstract: The objective of this study was to determine the effects of biotin supplementation on milk production and milk fatty acids in crossbred Holstein Friesian dairy cows. Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 64±45 days in milk, 13.0±2.4 kg of milk and 375±26 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 day^{-1} of biotin filled in a capsule and the third group was fed the same basal diet as the control group and supplemented with 40 mg day^{-1} of biotin filled in a capsule (BASF (Thai) Co., Ltd.). All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*; 35 day 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 fatty acid analysis. Rumen fluids were colleted on day 50 of the experiment at 0, 3, 5 and 7 h post feeding and then subjected to pH, ammonia nitrogen and volatile fatty acids. The results showed no significant differences in dry matter intake, live weight change, milk yield, milk compositions, milk fatty acids, ruminal pH, ammonia nitrogen and volatile fatty acids ($p>0.05$).

Key words: Biotin, milk production, milk composition, milk fatty acids, ruminal ammonia nitrogen, volatile fatty acids, 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.

In a controlled study added 0, 10 or 20 mg of biotin to the diet of cows 14 days before calving and during the first 100 days in milk (Zimmerly and Weiss, 2001). The significant increases in milk due to the addition of biotin were obvious 1 week after calving. The increase in milk averaged 0.9 and 2.7 kg day^{-1} during the 100 days period for 10 and 20 mg of biotin, respectively compared to the zero level of biotin. There were no differences in feed intake or milk composition but there was an increase in the yield of milk true protein. Additionally, the 20 mg of biotin

Corresponding Author: W. Suksombat, School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, University Avenue, Amphur Muang, 30000 Nakhon Ratchasima, Thailand

compared to zero biotin resulted in 1.6 kg more energy corrected milk. Majee *et al.* (2003) also in a controlled study with replicated 28 days periods showed a significant daily milk increase of 1.7 kg with 20 mg of added biotin. Dry matter intake increased 0.7 kg/cow and yield of milk fat and true protein were also significantly increased. When 40 mg of Biotin was compared to 20 mg there was no additional response.

Results of field trials with either zero or 20 mg of added biotin have resulted in milk yield increases of 0.9-2.7 kg day^{-1} for complete lactations in moderate to high producing cows. In a field trial by Bergsten *et al.* (2003) in which foot health was intensively monitored and feet were trimmed often to detect differences over entire lactations (170 cows), milk production numerically increased an average of 1.6 kg day^{-1} for cows receiving 20 mg vs. zero added biotin. The presence of subclinical foot lesions was greater at the end of the trial for zero compared to biotin supplementation even though the cows were on pasture 6 months of the year. The first lactation cows receiving 20 mg of added biotin had 61 fewer days from calving to conception and breeding per conception were 1.5 compared to 2.96 for the control cows.

Bergsten *et al.* (1999) conducted a controlled field trial with 180 cows in a commercial dairy herd fed supplements by computer feeder. Cows were supplemented with either 0 or 20 mg day^{-1} biotin during lactation over a 1 year period. Milk production was also increased in the biotin-supplemented cows, recorded as DHIA 305 days milk yield by 2.9 kg day^{-1}. The size of this increase led the authors to speculate that metabolic effects of biotin accounted for a significant portion of the milk production response. Zimmerly and Weiss (2001) fed dairy cows (n = 45) either 0, 10 or 20 mg supplemental biotin per day starting 14 days before calving through 100 days of lactation. Individual milk yield and dry matter intake was recorded daily. Cows fed 0, 10 or 20 mg biotin produced an average of 36.9, 37.8 and 39.7 kg of milk per day over the first 100 days of lactation. Biotin had a significant linear effect on milk production. The response to 20 mg biotin of +2.8 kg day^{-1} was similar to the increase reported by Bergsten *et al.* (1999) of +2.9 kg day^{-1} over 305 days of lactation. In this study the response to biotin was observed during the 1st week after calving and continued throughout the first 100 days of lactation. The rapid response indicated that biotin had a direct metabolic effect on milk production rather than a secondary effect via improved hoof health.

The dairy NRC does not provide recommended biotin allowances for lactating dairy cows because a lack of research on biotin requirements for milk production although, it was felt that in general, biotin requirements could be met through synthesis by rumen microorganisms and escape of basal dietary sources from the rumen. However, recent evidence of improve lactation performance from biotin supplementation can be found in the literatures. Thus, the present study aimed to determine the effects of biotin supplementation on milk yield, milk composition, live weight, live weight change, milk fatty acid, ruminal pH, ammonia nitrogen and volatile fatty acids.

MATERIALS AND METHODS

Animals and treatments: Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 64±45 days in milk, 13.0±2.4 kg of milk and 375±26 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 day^{-1} of biotin filled in a capsule and the third group was fed the same basal diet as the control group and supplemented with 40 mg day^{-1} of biotin filled in a capsule (BASF (Thai) Co., Ltd.). All cows also received *ad libitum* grass silage (*Brachiaria ruziziensis*; 35 days 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 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 fatty acid analysis: Milk samples were collected from individual cow on day 56 of the experiment. Milk samples of each period were centrifuged at 2000 g to fat cake and extraction. Lipid extraction was that of the procedures described by Hara and Radin (1978) using a volume of 18 mL of hexane and isopropanol (3:2, vol/vol)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% Na_2SO_4 in distilled H_2O) was added at a volume of 12 mL g^{-1} of fat cake. The hexane layer was transferred to a tube containing 1 g of Na_2SO_4 and after 30 min, the hexane layer was removed and stored at -20°C until methylation.

Fatty Acid Methyl Esters (FAME) were prepared by procedure described by Ostrowska *et al.* (2000). The procedure involved that approximately 30 mg of the extracted oil was placed into a 15 mL reaction tube fitted with a teflon-lined screw cap. About 1.5 mL of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. About 1 mL of C17:0 internal standard (2.00 mg mL^{-1} in hexane) and 2 mL of 14% boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking. After methylation was completed, 10 mL of deionized water was added. The solution was transferred to a 40 mL centrifuged tube and 5 mL of hexane was added for FAME extraction. The solution was centrifuged at 2000 g at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and was taken into vial for analyzed by Gas Chromatography (GC) (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 100 m×0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 240°C. The column temperature was kept at 70°C for 4 min then increased at 13°C min^{-1} to 175°C and held at 175°C for 27 min then increased at 4°C min^{-1} to 215°C and held at 215°C for 31 min.

Ruminal pH, NH_3-N and VFAS analysis: Ruminal fluid (100 mL) was collected on day 50 of the experiment from the ventral sac by suction via a polyethylene tube at 0, 3, 5 and 7 h post feeding. The pH of rumen fluid was immediately determined at the time of sampling by pH meter. Ruminal Volatile Fatty Acids (VFA) and ammonia N were determined from rumen fluid samples taken on 20 mL of rumen fluid and combined with 5 mL 6N HCl and kept at -20°C until analyzed for VFA and ammonia N. The samples were thawed at 4°C and centrifuged at 3,000 rpm for 15 min. The supernatant fluid was analyzed for ammonia N by Kjeldahl and VFA (acetate, propionate and butyrate) concentrations were determined by gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m× 0.25 mm×0.25 μm film (DB-FFAP).

Statistical analysis: Measurements of intake, milk production, milk composition, milk fattay acids and ruminal 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 (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Nutrient and fatty acid compositions of feeds used in the experiment are shown in Table 1 and 2, respectively.

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

Dry matter (%)	Concentrate	Grass silage
Dry matter	96.37	28.68
Crude protein	21.31	5.11
Crude fat	4.06	1.40
Ash	9.35	8.13
Crude fiber	12.31	36.24
Non fiber carbohydrate	26.20	14.70
Neutral detergent fiber	39.08	70.66
Acid detergent fiber	15.99	55.72
Acid detergent lignin	4.56	4.58
Neutral detergent insoluble nitrogen	1.10	0.12
Acid detergent insoluble nitrogen	0.39	0.17
TDN_{1X} (%)1	65.83	54.55
DE_P (Mcal kg^{-1})2	2.66	2.49
ME_P (Mcal kg^{-1})3	2.21	2.05
NE_{LP} (Mcal kg^{-1})4	1.39	1.21
Effective degradability of Dry Matter (dgDM)	60.90	37.30
Effective degradability of Crude Protein (dgCP)	69.40	48.20

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

Table 2: Fatty acid composition of feeds (% of total fatty acids)

Fatty acid profile	Concentrate	Grass silage
C14:0	4.98	2.07
C16:0	13.93	18.52
C18:0	2.71	4.86
C18:1n9c	23.65	4.59
C18:2n6c	17.93	9.88
C18:3n6	0.12	0.19
C20:0	0.66	0.93
C20:1n9	2.74	12.15
Others	33.22	46.70

Concentrate was rich in C18:1 and C18:2 while grass silage was rich in C20:1. Dry Matter (DM), Crude Protein (CP) and Net Energy for lactation (NE_{LP}) intakes of the experimental cows were similar ($p>0.05$) (Table 3). Similar results were previously reported when cows were supplemented with 20-30 mg day^{-1} 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 day^{-1}) when 20 mg day^{-1} 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 lactating dairy cows in early lactation with biotin (10 and 20 mg day^{-1}) did not affect DMI, milk yield, 3.5% fat-corrected milk yield and milk compositions (Table 3 and 4). Final live weight and live weight change were also not different across treatments. Weiss (2010) reviewed 1 from 5 research works published on the effects of supplemental biotin on lactating dairy cows (Bergsten *et al*., 1999; Fitzgerald *et al*., 2000; Midla *et al*., 1998; Zimmerly and Weiss, 2001). Two of the three field studies reported increased milk production when supplemental biotin was fed. The two positive studies used cows with high milk production (approximately 10,000 kg/305 days). The study that did not show increased milk production with supplemental biotin used low-producing cows (approximately 5,800 kg/305 days). Midla *et al*. (1998) and Zimmerly and Weiss (2001) reported a significant dose-dependent increase in milk and milk protein yield in cows supplemented with 10 or 20 mg day^{-1} biotin. Fitzgerald *et al*. (2000) supplemented pastured cows with 0 or 20 mg days^{-1} of biotin and found no significant difference in milk yield between treatment herds. The average milk production in the present study was low (11 kg day^{-1}) and it can be suggested that with higher production, the demand for biotin, required for biosynthesis of glucose, fatty acids and protein is increased. In the two studies in which milk production was increased in response to supplemental biotin, the average milk yield was 32 kg day^{-1} or greater. But in the Fitzgerald *et al*. (2000) experiment using lower producing cows (20 kg day^{-1}) no significant effect of biotin on milk yield was found. It can be suggested that supplementation of essential nutrients such as biotin will elicit an animal response when the supply of the nutrient is limiting in the metabolism (e.g., enzyme activity or other physiological processes of the animal). At higher levels of milk production, the activities of one or more biotin-containing enzymes may become limited by biotin supply or synthesis. Biotin is also a required for certain rumen bacteria and specifically for propionic acid synthesis (Baldwin and Allison, 1983) and may limit rumen metabolic activity under some conditions. It has also been shown that biotin synthesis by rumen organisms in continuous culture is reduced by increased proportions of grain in the ration (Abel *et al*., 2001). In the present study, no blood or milk samples were analyzed for biotin. In other studies, there was a significant increase in serum biotin in supplemented animals compared with those that were unsupplemented (Fitzgerald *et al*., 2000; Hedges *et al*., 2001; Zimmerly and Weiss, 2001). Another possible reason for the increase in milk production with biotin supplementation in some reports (Bergsten *et al*., 2003; Zimmerly and Weiss, 2001) includes improvements in DMI, hoof horn quality, energy balance, glucose production or utilization or cellulose digestion. When cows eat more they will be likely to produce more milk. In

Table 3: Effects of biotin supplementation on DM, CP and NELP intakes of dairy cows

		20	40		
Intake	Control	------(mg day^{-1})------		SEM	p-value
DM (kg)					
Concentrate	5.78	5.78	5.78		
Grass silage	5.39	5.56	5.17	0.26	0.58
Total	11.17	11.34	10.95	0.26	0.58
CP (g day^{-1})					
Concentrate	1232.00	1232.00	1232.00		
Grass silage	275.00	284.00	264.00	13.38	0.58
Total	1508.00	1516.00	1496.00	13.38	0.58
NE_{LP} (Mcal day^{-1})					
Concentrate	8.04	8.04	8.04		
Grass silage	6.52	6.73	6.26	0.31	0.57
Total	14.56	14.77	14.30	0.31	0.58

SEM = Standard Error of the Mean

Table 4: Effects of biotin supplementation on milk yield, milk composition, final liveweight and live weight change

		20	40		
Yields	Control	----(mg day^{-1})-----		SEM	p-value
Milk yield (kg day^{-1})	11.34	10.96	11.40	0.26	0.47
3.5% FCM (kg day^{-1})	12.13	12.04	12.62	0.30	0.37
Fat (%)	3.93	4.10	4.18	0.13	0.45
Protein (%)	2.74	2.81	2.76	0.10	0.89
Lactose (%)	4.30	4.27	4.29	0.04	0.90
Solid not fat (%)	8.04	8.04	8.09	0.14	0.96
Total solid (%)	11.97	12.14	12.26	0.26	0.73
Fat yield (g day^{-1})	446.00	450.00	474.00	15.40	0.38
Protein yield (g day^{-1})	310.00	305.00	312.00	11.50	0.90
Lactose yield (g day^{-1})	485.00	467.00	486.00	11.20	0.42
Solid not fat yield (g day^{-1})	911.00	880.00	920.00	22.40	0.43
Total solid yield (g day^{-1})	1357.00	1330.00	1394.00	34.50	0.43
Initial live weight (kg)	371.00	380.00	375.00	9.77	0.81
Final live weight (kg)	390.00	399.00	394.00	10.97	0.30
Live weight change (g day^{-1})	339.00	340.00	338.00	127.27	0.99

SEM = Standard Error of the Mean

this study, DMI was not different across treatments (11 kg day^{-1}) and therefore was not responsible for the increase in milk production.

Milk fat and protein percentages were unaffected by dietary biotin supplementation. Similar results were previously reported (Majee *et al*., 2003; Margerison *et al*., 2003; Zimmerly and Weiss, 2001). In contrast, yields of milk protein were higher in some reports (Majee *et al*., 2003; Zimmerly and Weiss, 2001) and Margerison *et al*. (2003) reported a higher yield of milk fat in the two continuous lactation studies (Margerison *et al*., 2003; Zimmerly and Weiss, 2001), there was no effect of biotin on dry matter intake. However, Majee *et al*. (2003) found intake was increased by 0.7 kg day^{-1}. Furthermore, biotin had no effect on body weight or condition score (Majee *et al*., 2003; Margerison *et al*., 2003; Zimmerly and Weiss, 2001).

Biotin supplementation had no effect on milk fatty acid composition in the present study although, biotin is involved in pathways for fatty acid synthesis (Table 5). However, C4:0, C18:2n6c and C20:1n9 tended to decrease when biotin was supplemented. Similar result (Enjalbert *et al*., 2008) was also obtained when biotin supplementation tended to decrease the proportion of fatty acids with <16 carbons at week 3 but the daily amount was not affected. Biotin tended to decrease biohydrogenation intermediates, increased C16:1 at week 3 and tended to increase cis-9 C18:1 at weeks 3 and 10. After 7 weeks of lactation, biotin tended to increase blood beta-hydroxybutyrate in multiparous cows with values remaining in a normal range and decreased plasma glucose in primiparous cows. These modifications of plasma parameters, milk protein content and profile of milk fatty acids could be due to a higher lipid mobilisation from adipose tissue driven by the increased milk production. Ruminal pH, NH_3-N and volatile fatty acids were also unaffected by biotin supplementation in this study (Table 6). Similar result was also reported (Zimmerly and Weiss, 2001) in which the study determined the effects of supplemental dietary biotin (0, 10, or 20 mg day^{-1}) on performance of Holstein cows (n = 45; 18 primiparous and 27 multiparous) and reported that molar proportions of ruminal volatile fatty acids were not affected by biotin supplementation. This suggests that that biotin does not affect fiber or diet digestibility. Furthermore, no effects on cow body weight or condition score have been reported which suggests biotin supplementation does not alter nutrient partitioning (Zimmerly and Weiss, 2001).

Table 5: Effects of biotin supplementation on fatty acid composition in milk (% of total fatty acid)

		20	40		
Fatty acid profile	Control	----------(mg day^{-1})----------		SEM	p-value
C4:0	2.000	1.290	1.990	0.130	0.06
C6:0	0.390	0.360	0.340	0.070	0.89
C8:0	0.930	0.860	0.830	0.060	0.56
C10:0	1.910	1.800	1.780	0.140	0.79
C11:0	0.020	0.030	0.070	0.020	0.49
C12:0	8.860	8.230	7.740	0.490	0.30
C13:0	0.060	0.060	0.070	0.005	0.19
C14:0	13.050	13.250	13.020	0.440	0.92
C14:1	1.880	1.690	1.210	0.320	0.35
C15:0	0.750	0.830	0.750	0.020	0.11
C16:0	30.580	31.160	30.630	0.590	0.74
C16:1	1.690	1.670	1.650	0.140	0.97
C18:0	9.720	10.780	10.610	0.830	0.63
C18:1n9t	2.260	2.060	2.230	0.120	0.44
C18:1n9c	23.820	24.360	25.350	0.930	0.51
C18:2n6t	0.063	0.075	0.065	0.007	0.56
C18:2n6c	1.280	1.010	0.890	0.110	0.06
C18:3n6	0.100	0.090	0.100	0.004	0.55
C20:0	0.170	0.210	0.230	0.010	0.11
C20:1n9	0.170	0.140	0.130	0.009	0.06
C18:3n3	0.100	0.090	0.100	0.003	0.55
C20:3n6	0.040	0.050	0.050	0.003	0.13
C22:0	0.050	0.060	0.060	0.007	0.73
C22:2	0.010	0.010	0.010	0.002	0.79
Short chain fatty acid	14.210	12.910	12.600	0.680	0.23
Medium chain fatty acid	47.970	48.630	47.160	0.950	0.56
Long chain fatty acid	37.800	38.760	39.550	1.350	0.66
Saturated fatty acid	68.600	68.690	68.110	1.640	0.86
Unsaturated fatty acid	31.100	31.850	31.880	1.110	0.85

SEM = Standard Error of the Mean

Table 6: Effects of biotin supplementation on pH, NH_3-N, VFAs and acetate:propionate ratio

	Level of biotin supplementation				
Hour after feeding	0 mg	20 mg	40 mg	SEM	p-value
pH					
Hour 0	7.00	7.10	7.40	0.193	0.75
Hour 3	6.50	6.50	6.90	0.164	0.66
Hour 5	6.60	6.40	7.00	0.214	0.64
Hour 7	6.60	6.70	7.10	0.173	0.55
NH_3-N		----------(mg dL^{-1})----------			
Hour 0	9.97	10.45	10.76	0.272	0.58
Hour 3	12.76	12.85	13.57	0.449	0.75
Hour 5	11.28	11.87	11.89	0.455	0.83
Hour 7	10.77	10.85	10.98	0.265	0.95
Acetate; C2		----------(mol/100/moL)----------			
Hour 0	76.57	76.62	76.76	0.157	0.88
Hour 3	76.84	77.14	76.51	0.164	0.44
Hour 5	76.15	76.67	77.03	0.514	0.80
Hour 7	76.16	77.16	77.31	0.168	0.18
Propionate; C3		----------(mol/100/moL)----------			
Hour 0	16.96	16.03	15.96	0.237	0.35
Hour 3	16.09	15.54	16.00	0.077	0.17
Hour 5	16.52	15.71	15.71	0.190	0.33
Hour 7	16.50	15.59	15.59	0.057	0.06
Butyrate; C4		----------(mol/100/moL)----------			
Hour 0	6.44	7.32	7.38	0.153	0.20
Hour 3	7.37	7.30	7.47	0.193	0.93
Hour 5	7.30	7.29	7.23	0.208	0.98
Hour 7	7.30	7.23	7.07	0.125	0.77
C2:C3					
Hour 0	4.52	4.78	4.81	0.076	0.41
Hour 3	4.77	4.96	4.78	0.035	0.23
Hour 5	4.61	4.90	4.90	0.093	0.48
Hour 7	4.62	4.95	4.62	0.053	0.06

SEM = Standard Error of the Mean

CONCLUSION

It can be clearly concluded in the present study that biotin supplementation had no effect on all parameters measured because cows, used in this experiment produced low milk yield. Thus, in low producing dairy cows, supplementation of biotin is not benefit.

ACKNOWLEDGEMENTS

Researchers would like to express special thanks to the University Dairy Farm, the Center for Scientific and Technological Equipment for their great support. Financial support was provided by the Institute of Research and Development, Suranaree University of Technology. Biotin was provided by BASF (Thai) Co., Ltd., Thailand.

REFERENCES

AOAC, 1998. Official Methods of Analysis. 16th Edn., AOAC Inc., Washington DC.

Abel, H., I. Immig, G.C. Cda and W. Steinberg, 2001. Research note: Effect of increasing dietary concentrate levels on microbial biotin metabolism in the artificial rumen simulation system (RUSITEC). Arch. Anim. Nutr., 55: 371-376.

Baldwin, R.L. and M.J. Allison, 1983. Rumen metabolism. J. Anim. Sci., 57: 461-477.

Bergsten, C., P.R. Greenough, J.M. Gay, R.C. Dobson and C.C. Gay, 2003. A controlled field trial of the effects of biotin supplementation on milk production and hoof lesions. J. Dairy Sci., 86: 3953-3962.

Bergsten, C., P.R. Greenough, R.C. Dobson and J. Gay, 1999. A controlled field trial on the effects of biotin supplementation on milk production and hoof lesions. J. Dairy Sci., 82: 34-34.

Enjalbert, F., M.C. Nicot and A.J. Packington, 2008. Effects of peripartum biotin supplementation of dairy cows on milk production and milk composition with emphasis on fatty acids profile. Livest. Sci., 114: 287-295.

Fitzgerald, T., B.W. Norton, R. Elliott, H. Podlich and O.L. Svendsen, 2000. The influence of long-term supplementation with biotin on the prevention of lameness in pasture fed dairy cows. J. Dairy Sci., 83: 338-344.

Hara, A. and N.S. Radin, 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem., 90: 420-426.

Hedges, J., R.W. Blowey, A.J. Packington, C.J. O'Callaghan and L.E. Green, 2001. A longitudinal field trial of the effect of biotin on lameness in dairy cows. J. Dairy Sci., 84: 1969-1975.

Majee, D.N., E.C. Schwab, S.J. Bertics, W.M. Seymour and R.D. Shaver, 2003. Lactation performance by dairy cows fed supplemental biotin and a B-vitamin blend. J. Dairy Sci., 86: 2106-2112.

Margerison, J.K., B. Winkler, G. Penny and A. Packington, 2003. The effect of biotin supplementation on milk yield, reproduction and lameness in dairy cattle. J. Dairy Sci., 86: 250-250.

Midla, L.T., K.H. Hoblet, W.P. Weiss and M.L. Moeschberger, 1998. Supplemental dietary biotin for prevention of lesions associated with aseptic subclinical laminitis (pododermatitis aseptica diffusa) in primiparous cows. Am. J. Vet. Res., 59: 733-738.

Ostrowska, E., F.R. Dunshea, M. Muralitharan and R.F. Cross, 2000. Comparison of silver-ion high-performance liquid chromatographic quantification of free and methylated conjugated linoleic acids. Lipids, 35: 1147-1153.

Rosendo, O., C.R. Staples, L.R. McDowell, R. McMahon and L. Adinga *et al*., 2004. Effects of biotin supplementation on peripartum performance and metabolites of Holstein cows. J. Dairy Sci., 87: 2535-2545.

SAS, 1996. SAS User Guide: Statistics. SAS Institute Inc., Cary, NC. USA.

Steele, G.D.R. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometric Approach. 2nd Edn., McGraw-Hill, New York.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in ration to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Weiss, B., 2010. Effect of supplemental biotin on performance of lactating dairy cows. Department of Animal Sciences. Ohio Agricultural Research and Development Center. The Ohio State University.

Zimmerly, A. and W.P. Weiss, 2001. Effects of supplemental dietary biotin on performance of Holstein cows during early lactation. J. Dairy Sci., 84: 498-506.