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Effect of Soybean Oil and Lactic Acid Bacteria Supplementation on Performance and CLA Accumulation in Milk of Dairy Cows

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Abstract: The objective of this study is to increase CLA content in dairy cow's milk and their performances through addition of lactic acid bacteria and soy bean oil in diets. Twenty four crossbred Holstein Friesian lactating dairy cows, averaging 22.6±5.7 kg milk day^{-1}, 96±5.5 days in milk and 457±54 kg body weight were blocked according to milk yield and days in milk. They were then randomly assigned into three treatments being control group, addition of *Lactobacillus plantarum* at 1×10^9 cfu/cow/day plus 200 g day^{-1} soy bean oil and addition of *Lactobacillus acidophilus* at 1×10^9 cfu/cow/day plus 200 g day^{-1} soy bean oil. The experiment was a Randomized Complete Block Design (RCBD). There were no significant differences in DMI, CPI, NELI, milk yield and milk composition ($p>0.05$). There were no significant differences ($p>0.05$) in CLA (cis-9, trans-11 and trans-10, cis-12 octadecadienoic acid) levels among the three groups. Thus, lactic acid bacteria addition had no effect on CLA concentration in milk. However, short-medium chain fatty acids and saturated fatty acids were significantly increased ($p<0.05$) by lactic acid bacteria addition. Furthermore, ruminal pH, volatile fatty acids including acetate, propionate, butyrate and acetate: propionate ratio were unaffected ($p>0.05$) by lactic acid bacteria addition. Lactic acid bacteria addition had no effect ($p>0.05$) on number of micro organisms in the rumen.

Key words: Conjugated linoleic acid, lactic acid bacteria, milk production, composition, soy bean oil, NELI

INTRODUCTION

Advance development of human consumption of fat begins after the finding of close relationship between saturated fatty acid consumption and abnormal problem in the body. There are campaigns to promote consumption of unsaturated fatty acids. Furthermore, medical researches support the role of unsaturated fatty acids on reduction in the risk of many diseases. Researchers found fat from seafood, containing a high n-3 unsaturated fatty acids, particularly Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). These two fatty acids play a major role in improving human's health status (Baer *et al*., 2001). Besides these fatty acids, there is another group of unsaturated fatty acid which has anticarcinogenic properties, Conjugated Linoleic Acid (CLA) which can be found in ruminant products (Chouinard *et al*., 2001). Conjugated linoleic acids are isomers of fatty acids found in small amount milk and meat of ruminants. Conjugated linoleic acid has been known to inhibit development of tumors in mice (Pariza and Hargraves, 1985). In addition, many researches reported that CLA could inhibit the development of tumor in fore stomach, mammary gland, lung and intestines of rats (Ha *et al*., 1990; Ip *et al*., 1991). Conjugated linoleic acid can be synthesized by rumen microbes, particularly *Butyrivibrio fibrisovens* from dietary fat through biohydrogenation process. CLA content in dairy cow's milk can be increased by supplementation of high linoleic acid raw materials. Donovan *et al*. (2000) who found an increase in CLA content in milk when supplemented with fish oil. Similarly, Dhiman *et al*. (1999) also found an increase in CLA content when supplemented with oil seeds or plant oils. Further, researches found that some bacteria can also produce CLA by converting linoleic acid added to media (Jiang *et al*., 1998; Lin *et al*., 1999; Alonso *et al*., 2003). In addition, Ogawa *et al*. (2001) found that *L. acidophilus* and *L. plantarum* can produce CLA *in vitro*. It is interested to study of the effect of lactic acid bacteria addition to dairy cattle diet on CLA content in milk would increase consumer's opportunity to receive CLA from milk and thus help to improve health.

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MATERIALS AND METHODS

Dairy cattle and feeding managements: About 24 crossbred Holstein Friesian lactating dairy cows, averaging 22.6±5.7 kg milk day^{-1}, 96±5.5 days in milk and 457±54 kg body weight were blocked according to milk yield and days in milk. They were then randomly assigned into three treatments of 8 cows in each group being control group, addition of *L. plantarum* at 1×10^9 cfu/cow/day plus 200 g day^{-1} soybean oil and addition of *L. acidophilus* at 1×10^9 cfu/cow/day plus 200 g day^{-1} soybean oil. The experiment was a Randomized Complete Block Design (RCBD).

Group 1: Eight cows received concentrate plus 200 g day^{-1} soybean oil (control).

Group 2: Eight cows received concentrate plus 200 g day^{-1} soybean oil and *L. plantarum* at 1×10^9 cfu/cow/day.

Group 3: Eight cows received concentrate plus 200 g day^{-1} soybean oil and *L. acidophilus* at 1×10^9 cfu/cow/day. The experiment lasted for 40 days including 10 days for adjustment period followed by six five days periods for measurements.

Feed intake and dairy cow's performances: Feed offered and left uneaten were sampled on 2 consecutive days in each period of six 5 days periods. Samples were then dried at 60°C until dry and were ground through 1 mm sieve. Samples taken were subjected to several analysis as follow; proximate analysis (CP, DM, EE and ash) (AOAC, 1990); detergent analysis (NDF, ADF and ADL) (Georing and van Soest, 1970) and fatty acids in the diets by Gas chromatography. Milk yields were recorded daily while milk samples were taken on 2 consecutive days in each period of six five days periods. They were then analyzed for fat, protein, lactose, SNF and total solid by MilkoScan S50. On day 10, 20 and 30 of the experimental period, milk samples were taken to analyze for fatty acids and CLA (gas chromatography; Hewlett Packard GCD system HP 6890).

Analysis of fatty acid by Gas Chromatography (GC): Fatty acid analysis was carried out as previous described (Hara and Radin, 1978). In brief, milk fat was extracted from milk using hexane and isopropanol (3:2, vol/vol)/g of fat cake, modified from Kelly *et al*. (1998). Heptadecanoic acid (17:0) was added as an internal standard. The Fatty Acid Methyl Esters (FAME) were analyzed by GC (HP 6890, Hewlett Packard, USA) using a CP-Sil88 column for FAME (100 m×250 μm) (Chrompack, The Netherlands). The GC conditions were as follows: injected temperature, 240°C; detector temperature, 260°C; carrier gas, He; Split ratio, 1/30; temperature program, 70°C for 4 min followed by an increase of 13°C min^{-1} to 175°C then 4°C min^{-1} to 215°C. Peaks were identified by comparison of retention times with those of the corresponding standards (SupelcoTM 37 component FAME Mix, Sigma-Aldrich Co., USA).

The study of microorganism population in the rumen: Six fistulated non-lactating dairy cows were used to determine population of rumen microorganisms. The experimental design was 3×3 replicated latin square designs. The control diet was added 200 g day^{-1} soybean oil. Cows were allowed 2 weeks for adjustment period followed by 7 days period of measurement. At the end of each collected period, rumen samples were collected through the fistula at 6 h post feeding. The pH of the rumen fluid was immediately determined at the time of sampling by pH meter. Ruminal Volatile Fatty Acids (VFA) 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. The samples were thawed at 4°C and centrifuged at 3,000 rpm for 15 min. The supernatant fluid was analyzed for 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 mm film (DB-FFAP). Digesta from the rumen were collected 6 h after morning feeding (Ghorbani *et al*., 2002). Samples were plated on 4 different media; PDA (Potato Dextrose Agar), Rogosa, Streptococcus selective agar and E-medium for anaerobes for plate count.

Statistical analysis: Measurements of intakes, milk production, pH, NH_3-N and VFAs were subjected to analysis of variance using PROC GLM and the differences between means were subjected to orthogonal comparison using the Statistical Analysis System (SAS, 1988).

RESULTS AND DISCUSSION

Chemical composition of concentrate and grass silage used in the experiment is shown in Table 1. An evaluation of energy concentration in concentrates and grass silage showed that concentrates and grass silage contained 70.79 and 46.56% TDN1x, 3.58 and 2.34 Mcal kg^{-1} DM Dep, 3.18 and 1.92 Mcal kg^{-1}DM Mep and 2.05 and 1.15 Mcal kg^{-1} DM NELp, respectively. Fatty acid composition of concentrate, grass silage and soybean oil are showed in Table 2. Soybean oil contains high level of linoleic acid. Table 3 showed feed consumption of the

experimental cows. Concentrate DM intake of the three groups was similar at 9.6 kg day^{-1} while grass silage DM intakes were 5.3, 5.9 and 5.4 kg day^{-1} for cows on control, *L. plantarum* and *L. acidophilus*, respectively. Milk yield and milk composition are shown in Table 4. There were no significant differences (p>0.05) in milk yield and milk composition yield, percent fat, percent protein, percent lactose, percent solid not fat and percent total solid. Initial and final live weights were similar (p>0.05) in all treatment groups (Table 4). However, there were significant differences (p<0.05) in live weight changes. Cows on both strain of lactic acid bacteria addition lost weight while cows on the control feed gained weight. Fatty acid compositions in dairy cow's milk of the three groups are shown in Table 5.

Lactic acid bacteria supplementation significantly increased $C_{6:0}$ (p<0.01) compared to the control while *L. plantarum* addition significantly increased $C_{6:0}$ (p<0.05) compared to *L. acidophilus*. $C_{10:0}$ short chain fatty acids were significantly increased (p<0.05) by lactic acid bacteria supplementation compared to the control group.

Total short chain fatty acids were also significantly increased (p<0.05) by lactic acid bacteria addition. However, there were no significant differences (p>0.05) in other fatty acids when compared to the control cows. CLA (cis-9, trans-11 and trans-10, cis-12 octadecadienoic acid) was not significantly different (p>0.05) among treatment groups due to lactic acid bacteria addition. However, medium and long chain fatty acids were significantly increased (p<0.05) when lactic acid bacteria were included in the diet. Levels of rumen pH at various hours after feeding of experimental cows are shown in Table 6. After feeding, pH in the rumen measured from rumen fluid decrease as the time after feeding increased up to 5 h and then gradually increases. However, there were not statistically different (p>0.05) among treatments. Table 6 shows concentrations of acetate, propionate, butyrate and A:P ratio in the rumen.

Concentration of acetate, propionate, butyrate and A:P ratio were similar (p>0.05) in all treatments by lactic acid bacteria addition. The numbers of microorganisms in the rumen of fistulated cows received control diet and *Lactobacilli* sp. The microorganisms measured were Lactobacilli, Clostridia, Yeast and Mold, Streptococci and

Table 1: Chemical compositions of feeds used in the experiment

Chemical composition	Dry matter (%) Concentrate	Grass silage
Dry matter	94.57	24.86
Crude protein	20.91	9.25
Ether extract	6.31	2.08
Ash	7.87	10.94
Crude fiber	11.46	36.71
Neutral detergent fiber	41.76	68.38
Acid detergent fiber	16.52	46.96
Acid detergent lignin	3.94	7.59
Neutral detergent insoluble N	0.97	0.45
Acid detergent insoluble N	0.79	0.52
TDN_{1x} (%)[1]	70.79	46.56
DE_P (Mcal kg^{-1} DM)[2]	3.58	2.34
ME_P (Mcal kg^{-1} DM)[3]	3.18	1.92
NE_{LP} (Mcal kg^{-1} DM)[4]	2.05	1.15

[1]TDN_{1X} (%) = tdNFC+tdCP+(tdFA×2.25)+tdNDF – 7, [2]DE_{1X} (Mcal kg^{-1}) = [(tdNFC/100) ×4.2]+[(tdNDF/100) ×4.2]+[(tdCP/100)×5.6]+[(FA/100) ×9.4] – 0.3, Discount = [(TDN_{1X} – [(0.18×TDN_{1X}) – 10.3]) × Intake)]/TDN_{1X}, DE_P (Mcal kg^{-1} DM) = DE_{1X} × Discount, [3]ME_p = [1.01× (DE_p) – 0.45]+[0.0046× (EE – 3)], [4]NE_{Lp} = ([0.703× Me_p (Mcal kg^{-1})] – 0.19)+([(0.097×ME_p+ 0.19)/97] × [EE – 3])

Table 2: Fatty acid composition of feeds and soybean oil

Items	Total fatty acid (%) Concentrate	Grass silage	Soybean oil
$C_{14:0}$	5.24	0.75	0.09
$C_{16:0}$	13.25	20.57	10.68
$C_{18:0}$	3.13	2.78	4.22
$C_{18:1n9c}$	24.57	4.26	22.41
$C_{18:2n6c}$	31.65	13.21	54.74
$C_{20:0}$	0.43	1.59	0.39
$C_{18:3n6}$	0.08	0.00	0.32
$C_{20:1}$	3.38	15.45	6.58
$C_{22:0}$	0.32	1.64	0.42
$C_{24:0}$	-	0.51	0.15
Others	17.94	39.24	-

Table 3: Dry matter, crude protein and net energy for lactation intakes of the experimental cows

Items	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	Contrast 1 vs. 2 and 3	Contrast 2 vs. 3
DM intake (kg DM)						
Concentrate	9.60	9.60	9.60	-	-	-
Roughage	5.30	5.70	5.40	0.20	0.28	0.13
Total	14.90	15.30	14.90	0.22	0.27	0.12
CP intake (g day^{-1})						
Concentrate	1941.00	1941.00	1941.00	-	-	-
Roughage	584.00	614.00	585.00	10.52	0.29	0.10
Total	2525.00	2544.00	2526.00	10.86	0.30	0.11
NE_{LP} intake (Mcal day^{-1})						
Concentrate	19.55	19.55	19.55	-	-	-
Roughage	6.17	6.59	6.21	0.24	0.28	0.12
Total	25.72	26.15	25.76	0.25	0.28	0.12

Table 4: Milk yield and milk composition and body weight and BWC of the experimental cows

Items	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	Contrast 1 vs. 2 and 3	Contrast 2 vs. 3
Milk yield (kg day^{-1})	18.50	19.20	18.20	1.34	0.86	0.47
FCM (3.5%)	17.80	18.90	19.00	1.49	0.38	0.92
Fat (%)	3.27	3.44	3.79	0.29	0.19	0.23
Protein (%)	2.69	2.63	2.63	0.09	0.45	0.98
Lactose (%)	4.51	4.50	4.60	0.10	0.60	0.32
SNF (%)	8.10	8.05	8.15	0.16	0.98	0.52
Total solid (%)	11.38	11.49	11.95	0.36	0.28	0.21
Fat yield (g day^{-1})	603.60	652.10	686.00	66.55	0.26	0.61
Protein (g day^{-1})	488.80	501.40	476.50	30.87	0.99	0.42
Lactose (g day^{-1})	828.50	857.50	837.90	64.23	0.73	0.76
SNF (g day^{-1})	1483.00	1534.50	1482.00	103.50	0.78	0.61
Total solid (g day^{-1})	2087.00	2186.50	2186.00	154.00	0.50	0.90
Final weight	456.00	442.00	440.00	25.38	0.49	0.96
BWC (g day^{-1})	17.90	-317.00	-607.00	264.70	0.04	0.28

BWC = Body Weight Change

Table 5: Fatty acid composition of milk fat of the experimental cows

Items	Milk fat (mg g^{-1}) Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	Contrast 1 vs. 2 and 3	Contrast 2 vs. 3
$C_{4:0}$	21.74	22.81	23.230	1.25	0.38	0.39
$C_{6:0}$	11.26	12.95	13.850	0.63	0.01	0.01
$C_{8:0}$	7.70	6.77	7.840	1.28	0.59	0.47
$C_{10:0}$	11.55	13.75	20.520	4.29	0.04	0.61
$C_{11:0}$	1.21	1.39	1.510	0.16	0.14	0.26
$C_{12:0}$	37.79	41.64	41.330	2.60	0.48	0.15
$C_{13:0}$	1.11	1.14	1.580	0.33	0.46	0.16
$C_{14:0}$	63.08	78.84	78.890	5.29	0.09	0.74
$C_{14:1}$	10.59	9.54	14.140	3.23	0.16	0.74
$C_{15:0}$	4.93	4.99	5.090	0.31	0.63	0.83
$C_{16:0}$	179.58	188.50	197.620	11.08	0.17	0.43
$C_{16:1}$	20.46	22.36	22.060	2.13	0.72	0.38
$C_{18:0}$	73.58	80.28	88.290	8.89	0.15	0.46
$C_{18:1n9t}$	31.17	23.26	38.231	9.75	0.20	0.42
$C_{18:1n9c}$	293.74	306.86	291.200	16.62	0.53	0.43
$C_{18:2n6t}$	2.81	2.52	2.310	0.41	0.32	0.48
$C_{18:2n6c}$	25.04	25.22	24.190	1.89	0.26	0.92
$C_{20:0}$	2.70	1.24	2.120	1.06	0.86	0.18
$C_{18:3n6}$	1.30	1.32	1.230	0.09	0.29	0.88
CLAa[1]	6.86	6.76	7.010	0.61	0.70	0.87
CLAb[2]	0.13	0.06	0.060	0.05	0.39	0.17
$C_{22:0}$	0.41	0.37	0.450	0.05	0.18	0.40
$C_{20:3n6}$	0.91	0.93	1.070	0.09	0.20	0.19
$C_{22:1n9}$	0.79	0.67	0.780	0.08	0.48	0.14
Short[3]	92.34	100.88	109.410	5.57	0.01	0.14
Medium[4]	278.64	304.23	317.790	15.54	0.02	0.39
Long[5]	449.51	449.70	442.480	12.58	0.66	0.42
Saturated	413.50	453.50	475.440	22.52	0.01	0.34
Unsaturated	406.99	401.32	394.240	21.30	0.57	0.64

[1]CLAa = cis-9, trans-11 octadecadienoic acid, [2]CLAb = trans-10, cis-12 octadecadienoic acid, [3]Short chains FA: ($C_{4:0}$-$C_{13:0}$), [4]Medium chains FA: ($C_{14:0}$-$C_{17:0}$), [5]Long chains FA: ($\geq C_{18:0}$)

protozoa. The results showed that supplementation of lactic acid bacteria had no effects on number of micro organisms in the rumen ($p>0.05$). Chemical composition analysis showed higher fat content and energy in soybean oil supplementation groups and has 86% true digestibility (NRC, 2001). Estimates of TDN_{1X}, DE_P and NE_{LP} in oil supplemented concentrate therefore were relatively high. The present study found no significant differences in DM, CP and NE_L consumptions among treatment groups. Milk yields and fat corrected milk yields were unaffected by bacteria addition, although bacteria addition cows tended to produce 0.9-1.2 kg day^{-1} higher fat corrected milk than the control cows. This is consistent with the finding of Jaquette *et al.* (1988) and Ware *et al.* (1988) who found that milk yield increased when 1×10^9 cfu/cow/day *L. acidophilus* were added to the diet. Jeong *et al.* (1998) also found an increase in 0.8 kg day^{-1} milk yield when *Lactobacillus* sp. was

Table 6: Concentrations of volatile fatty acids, pH and bacteria and protozoa population in ruminal fluidof experimental cows

Items	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	Contrast 1 vs. 2 and 3	Contrast 2 vs. 3
pH level						
0 h	6.64	6.54	6.66	0.10	0.40	0.37
2 h	6.34	6.31	6.47	0.16	0.33	0.84
4 h	6.33	6.22	6.40	0.17	0.35	0.48
6 h	6.36	6.20	6.35	0.14	0.41	0.13
VFAs mol/100 mol						
Acetate	73.17	75.15	75.27	1.41	0.51	0.32
Propionate	15.49	18.39	15.98	1.11	0.42	0.06
Butyrate	5.66	6.33	6.89	1.04	0.27	0.43
A:P	4.87	4.17	4.75	0.34	0.54	0.12
Grouping bacteria	----------Digesta ($\times 10^6$ cfu g^{-1})----------					
Lactobacilli	1.72	1.60	1.79	0.18	0.47	0.52
Clostridia	1.66	1.78	1.96	0.29	0.24	0.57
Yeast + Mold	1.25	1.03	1.00	0.22	0.64	0.24
Streptococci	1.52	1.55	1.20	0.54	0.13	0.89
Protozoa ($\times 10^5$ mL^{-1})	3.20	2.60	3.21	0.56	0.48	0.23

included in the diet. Increases in milk yield reflected higher lactate produce and thus, propionate produced when lactic acid bacteria were added to the diet. Propionate has been known to be precursors for glucose synthesis and thus lactose synthesis in mammary glands. Higher lactose was synthesized, higher milk yield was produced. The $C_{6:0}$ and $C_{10:0}$ fatty acids were increased when lactic acid bacteria were added to the diet. Although, acetate and butyrate contents in the rumen were not statistically different between supplemented and unsupplemented groups, they were numerically higher in lactic acid bacteria supplemented groups. Short and medium chain fatty acids were subjected to de novo synthesis in mammary glands from acetate (Banks *et al*., 1984; Grummer, 1991; Palmquist *et al*., 1993). A tendency towards increases in acetate resulted in increases in short ($C_{4:0}$-$C_{13:0}$) and medium ($C_{14:0}$-$C_{17:0}$) chain fatty acids and saturated fatty acids in milk.

After feeding, pH in the rumen measured from rumen fluid decreased as the hour after feeding increased up to 6 h then slightly increased. Levels of pH in the rumen below 5.9 can cause rumen acidosis (Seal and Parker, 1994; Garrett *et al*., 1999). The lowest rumen pH in the experiment was at 6 h after feeding and was >5.9. Feeding 1×10^9 cfu/cow/day lactic acid bacteria had no toxic effect on rumen pH. Kim *et al*. (2000) also found unchanged rumen pH when fed *P. acidipropionici* and *L. plantarum* to the cows. In contrast, Nocek *et al*. (2000) found reduction in rumen pH up to 5.5 when Enterococcus and Lactobacillus were fed to the cows. This will cause a risk of sub clinical ruminal acidosis. Concentrations of acetate, propionate, butyrate and A:P ratio in the rumen. Concentration of acetate, propionate, butyrate and A:P ratio were similar ($p>0.05$) in all treatments by lactic acid bacteria addition in the experiment. Similarly, Kim *et al*. (2000) observed increases in propionate when lactate-producing and utilizing bacteria (*L. plantarum* and *P. acidipropionici*) were fed. Acetate:Propionate (A:P) <2.2:1 can cause rumen acidosis. The experiment observed acetate:propionate ratios of all cows were in the range of 2.99-3.32 which were higher than the risk level. Garrett *et al*. (1999) suggested that when ruminal pH reduced to below 5.9, acetate: propionate ratio dropped below 2.2:1. The results of the experiment showed that supplementation of lactic acid bacteria had no effects on number of microorganisms in the rumen. Boyaval *et al*. (1995) found that linoleic acid had a negative effect on growth and metabolism of bacteria. Soybean oil was included in concentrate in the experiment since, it contained high amount of long chain fatty acids. These fatty acids probably inhibit the growth and metabolism of the microorganisms in the rumen. Galbraith and Miller (1973a, b) reported that unsaturated fatty acids can inhibit cell respiration and thus cause cell lysis.

CONCLUSION

The present study revealed that lactic acid bacteria supplementation had no effect on CLA content of milk, DM, CP and NE_L consumptions, milk yield and milk compositions. However, lactic acid bacteria inclusion in the diet significantly increased short and medium chain fatty acids and saturated fatty acids in milk while long chain fatty acids and CLA content in milk, rumen pH, VFAs and microorganism population in the rumen were unaffected by the addition of lactic acid bacteria.

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