Asian-Aust. J. Anim. Sci.
Vol. 22, No. 11 : 1504 - 1512
November 2009

www.ajas.info

Milk Conjugated Linoleic Acid (CLA) Profile and Metabolic Responses of Dairy Cows Fed with High-temperature-micro-time (HTMT) Treated Diets Containing High Quantity Extruded Soybean (ESB)*

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ABSTRACT : A feeding trial was conducted to examine the effect of high-temperature-micro-time (HTMT) processing of diets containing extruded soybean (ESB) in high quantity on milk fat production, metabolic responses, and the formation of conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA). Twenty-one multiparous Holstein cows in mid-lactation were blocked according to milk yield in the previous lactation. Cows within each block were randomly assigned to either normal concentrate or HTMT treated diets containing ESB (7.5% HTMT-ESB and 15% HTMT-ESB). It was hypothesized that the HTMT-ESB would affect the undegradable fatty acids in the rumen and, thus, would modify the fatty acid profile of milk fat. Both 7.5% and 15% HTMT-ESB did not affect milk yield, fat, protein, lactose and solid-not-fat (SNF), but the proportion of *cis*-9, *trans*-11 CLA in milk fat was significantly increased by these treatments. Content of TVA in milk fat was not affected by HTMT-ESB. The HTMT-ESB influenced the fatty acid profile in milk fat, but there was little difference between 7.5% and 15% of supplementation. HTMT-ESB feeding significantly decreased the concentration of plasma insulin and glucose, while plasma growth hormone (GH), triglyceride (TG), non-esterified fatty acid (NEFA) and HDL-cholesterol were increased by 7.5% and 15% ESB-HTMT supplementation in comparison to the control group ($p<0.05$). However, no significant difference was observed in plasma LDL-cholesterol, insulin like growth factor (IGF)-1, T3, T4, and leptin concentrations among treatments ($p>0.05$). The present results showed that *cis-9, trans-11* CLA production was increased by HTMT treatment of dietary ESB without reduction of milk fat, and the unchanged milk fat and yield was assumed to be associated with the constant level of thyroid hormones, leptin, and IGF-1. (**Key Words :** HTMT, Milk Fat, CLA, Metabolic Response, Holstein Cow)

INTRODUCTION

Free unsaturated fatty acids released from the degradation of dietary lipids in the rumen are subjected to bio-hydrogenation by the rumen microorganisms (Harfoot and Hazlewood, 1988). *Cis*-9, *trans*-11 conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA, *trans*-11 $C_{18:1}$) are formed as a result of incomplete bio-hydrogenation of unsaturated fatty acids (USFA). It is known that the contents of CLA and TVA tend to be positively correlated in rumen contents (Bessa et al., 2000). CLA has received growing attention due to its beneficial health effects such as the suppression of carcinogenesis (Belury, 1995; Lee et al., 2004), obesity (Park et al., 1997), artherogenesis (Nicolosi et al., 1997), and diabetes (Houseknecht et al., 1998).

Full-fat soybeans, which contain 40% crude protein (CP) and 18% fat, are good sources of protein and energy for the ration of high-yielding dairy cows. The role of supplementary fat in dairy rations has been previously reviewed (Palmquist, 1988). Previous studies demonstrated that the milk fat concentration of CLA was dependent on

* This work was supported by Brain Korea 21 program and research grants BioGreen 21 Program (20050401-034-701-136-0300), Rural Development Association, Republic of Korea.
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Received June 22, 2007; Accepted April 10, 2009

the amount of dietary unsaturated fatty acids (Griinari et al., 1996), and that CLA concentration was correlated to the dietary level of oil or oilseed (Bateman and Jenkins, 1998; Wang and Song, 2001; Duckett et al., 2002).

In the ruminant, however, full-fat soybean, a high dietary plant oil source, has a negative effect on rumen microbial growth (Jenkins, 1993). Furthermore, the TVA induced by USFA effectively inhibited fatty acid synthesis in the mammary tissue (Gaynor et al., 1994). To overcome these problems, heat treatment of the diet has been conducted. Several studies reported that the processing of oilseed supplements could influence the degree and extent of bio-hydrogenation (Murphy et al., 1995), and thus caused a more efficient increase in the CLA content of milk fat compared with supplementation trials without processing of an oilseed diet containing linoleic acid-rich oil (Dhiman et al., 1999; Chouinard et al., 2001). In these previous studies, however, the method of processing oil supplements decreased milk fat percentage when cows were fed a heat-treated diet with corn silage as the predominant dietary source (Block et al., 1981; Van Dijk et al., 1983; Guillaume et al., 1991).

In the present study, the experimental diet was obtained by a high-temperature-micro-time (HTMT) processing method, which increases bound fat content in the diet. This treatment was thought to decrease the releasing rate of the lipid from bound fat, and an increase in CLA production in the rumen without negative impacts on rumen fermentation. Therefore, it could be hypothesized that HTMT treatment may improve milk production without depression of milk fat.

Thus the objectives of the present study were i) to determine the effect of HTMT-treated diets containing ESB on milk fat production and milk fatty acid profiles, especially CLA and TVA, and ii) to examine lipid-related metabolic changes when cows were fed HTMT-ESB.

MATERIALS AND METHODS

Animals and diets

Twenty-one multiparous Holstein cows (653.4±22.25 kg) in mid-lactation were blocked according to milk yield in the previous lactation. Cows within each block were randomly assigned to either normal concentrate or HTMT-ESB concentrate, treatment 1 with 7.5% ESB supplementation and treatment 2 with 15% ESB supplementation. The ESB contained 18% fat, consisting of 51% of C18:2 in total fatty acids, and were of interest as a source of protein and energy in the ration of high yielding dairy cows. In the present study, the 7.5% and 15% of ESB was supplemented to feed concentrates in treatments 1 and 2, respectively. Although the concentrate used in treatment 1 contained higher rapeseed meal and tallow -5.82% and 1.95%, respectively, for treatment 1, while 0.8% and 0%, respectively, for treatment 2. There was no significant difference in ether extract content between the two treatments (Tables 1 and 2). Concentration of $C_{18:2}$(linoleic acid), $C_{18:3}$(linolenic acid) and USFA in experimental diets was 1.93 and 2.53; 0.18 and 0.27; 3.71 and 4.50 g/100 g fat for treatments 1 and 2, respectively. Concentrations of linoleic acid were higher than the control (1.32, 0.08, and 2.92 g/100 g fat). The composition of C_{18}-fatty acids in ESB, rapeseed and tallow are presented in Table 3. Cows were fed diets containing corn silage as forage and concentrate in a 50:50 ratio that was formulated to meet the nutrient demand of cows according to NRC (1989). All the test diets had similar CP content. Additional ESB increased the total ether extract content in diets of treatments 1 and 2 by approximately 1.4-1.7% unit compared with the control.

The HTMT treatment was conducted using a Universal Pellet Cooker (UPC, Wenger Manufacturing, Inc., Sabetha, KS, USA) at 125-170°C for 3-4 seconds. Ingredients and chemical composition of experimental diets are shown in Table 1 and 2. Corn silage was used as a forage source. The dry matter (DM) percentage of corn silage (crude protein (CP): 1.81%/DM, ether extract (EE): 0.53%/DM, crude fiber (CF): 6.56%/DM, Ash: 1.47%/DM, Ca: 0.03%/DM, P: 0.05%/DM and total digestible nutrients (TDN): 11%/DM) and concentrate was determined weekly, and the total mixed diets were adjusted to maintain constant forage to concentrate ratio of 50:50 on a DM basis. The rations were fed twice daily at 0830 h and 1700 h.

The diets were formulated according to NRC (1989) guidelines for milk production at 30 kg/d with 3.8% of milk fat. Cows had free access to water and mineral block. Random grab samples of the concentrate were ground through a 1-mm screen for the analyses of DM, EE, CP, CF and TDN, using the AOAC (1984) method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Van Soest et al. (1970) and AOAC (1990). The experimental period consisted of 10 weeks of which the first 7 days were used as an adjustment period. All animal-based procedures were in accordance with the "Guidelines for the Care and Use of Experimental Animals of Seoul National University", formulated from the "Declaration of Helsinki and Guiding Principles in the Care and Use of Animals."

Sampling and analysis of milk

Feed intake and milk yield were recorded daily. Milk samples were collected from three consecutive morning and afternoon milkings during the final 7 days of the experiment. Milk samples were preserved with bronopol-B2 and stored at 4°C until further analysis. Preserved milk samples were analyzed for fat, protein, lactose and solid non-fat content

Table 1. Ingredient composition of experimental concentrates

Items	Control	7.5% ESB-HTMT[1]	15% ESB-HTMT[2]
Ground yellow corn	14.00	13.23	12.45
Extruded soy bean	0.00	7.50	15.00
Ground wheat	10.00	10.00	10.00
Gluten feed	9.00	4.77	0.53
Corn flour	5.00	5.00	5.00
Corn germ meal	5.15	5.08	5.00
Rice bran	1.98	2.49	3.00
Soy hulls	7.65	7.63	7.61
Lupin hulls	7.00	6.00	5.00
Wheat flour	3.00	3.00	3.00
Wheat bran	1.50	4.24	6.97
Copra meal	5.00	5.00	5.00
Cottonseed meal	10.00	10.00	10.00
Rapeseed meal	9.69	5.82	1.95
Gypsum	0.11	0.08	0.05
$CaCO_3$	1.07	1.20	1.32
Defluorinated phosphorus	0.78	0.69	0.60
Salt	1.00	1.00	1.00
Urea	0.25	0.28	0.30
Tallow	1.60	0.80	0.00
Molasses	6.00	6.00	6.00
Mineral mix [3]	0.16	0.16	0.16
Vit. mix[4]	0.06	0.06	0.06
Total	100	100	100

[1] HTMT-treated diet containing 7.5% ESB. [2] HTMT-treated diet containing 15% ESB.
[3] Contained 5.0% Mg, 7.0% K, 9. 5% S, 3.0 Zn, 2.5% Mn, 2.0% Fe, 0.02% Se, and 0.005% Co, others.
[4] 2,300 IU/g of vitamin A, 650 IU/g of vitamin D, and 8 IU/g of vitamin E.

Table 2. Chemical composition of experimental concentrates

Item	Control	7.5% ESB-HTMT	15% ESB-HTMT
Chemical composition	---------- % of dry matter ----------		
Crude protein	19.22	19.70	19.70
Ether extract	2.90	4.31	4.56
Acid detergent fiber	13.60	13.55	13.74
Neutral detergent fiber	23.70	23.70	22.93
Rumen degradable protein	13.45	13.45	13.50
Rumen undegradable protein	6.21	6.25	6.20
Crude fiber	12.10	10.50	10.50
Calcium	0.80	0.90	0.90
Non structure carbohydrate	36.20	35.00	35.00
Total digestible nutrient	72.13	73.29	73.30
Fatty acid	---------- g/100 g of total fatty acid ----------		
18:2n6 (linoleic acid)	1.32	1.93	2.53
18:3n3 (linolenic acid)	0.08	0.18	0.27
USFA[1]	2.92	3.71	4.50
n3 PUSFA[2]	0.08	0.18	0.27
Total N6 PUFAS[3]	1.33	1.94	2.54

[1] Unsaturated fatty acid. [2] n3- polyunsaturated fatty acid. [3] n6- polyunsaturated fatty acid.

Table 3. C18-fatty acid composition of major lipid sources in experimental diets

Fatty acids	Extruded soybean	Rapeseed	Tallow
	---------- g/100 g of Total fatty acid ----------		
C18:0	3	4	16
C18:1	22	44	43
C18:2	51	29	4.3
C18:3	7.5	13	0.5

by infrared-aided procedures with a Milkscan-133B, using a B filter. Weighed composite milk samples from morning and afternoon milking were analyzed for fatty acid composition, including CLA, using the following procedures after fat was extracted using the method of Folch et al. (1957). Methylation of fatty acids followed the method of Lepage and Roy (1986) prior to the GC analysis. A fused silica capillary column (100 m×0.25 mm, i.d., 0.20 μm thickness, SPTM-2560, Supelco) was used. The initial column temperature was 175°C held for 30 minutes, and then increased by 15°C/min to 220°C held for 40 minutes. Ultra pure helium was used as a carrier gas. Fatty acids were identified by comparing the retention times with methylated fatty acid standards including CLA (Sigma Chemical Co., St. Louis, MO). The reported CLA isomer was the total isomers. The percentage of each fatty acid was calculated by dividing the area under the fatty acid peak by the sum of the areas under the total reported fatty acid peaks.

Sampling and analysis of blood

Blood samples of 20 ml were taken from the jugular vein at 1 h before the morning feeding at 0800 h on day 70 for the analysis of plasma triglyceride (TG), non-esterified fatty acid (NEFA), LDL-cholesterol, HDL-cholesterol, insulin, growth hormone (GH), and insulin-like growth factor (IGF)-1. Blood was collected into evacuated test tubes (Vacutainer; Becton Dickinson Vacutainer Systems, Rutherford, NJ, USA) containing sodium heparin. The collected blood was centrifuged, and plasma was stored at -70°C until used for assay.

Plasma NEFA and plasma glucose were, respectively, measured by the acyl-CoA synthase acyl-CoA oxidase method (NEFA-C, Wako Chemical, Japan) and the glucose oxidase method (Glucose CII-test Wako Chemical, Japan), using commercial kits. TG, HDL-cholesterol and LDL-cholesterol were measured by the Homogeneous Enzymatic Colorimetric Method using TG-C plus, HDL-C plus, and LDL-C plus kits (Roche, USA).

Plasma insulin was measured by sandwich enzyme immunoassay using a commercial kit (Elisa Auto Insulin, International Reagents Cooperation, Japan). Plasma GH and IGF-1 were assayed by radioimmunoassay (RIA) as previously described by Lee et al. (2000). Briefly, plasma GH was assayed by a double antibody method using bovine anti serum (USDA-anti-bGH, lot AFPB55) and bovine GH (USDA-bGH, lot AFP-11182) was used for GH standard and iodinated by the chloramine T method. The sensitivity was 0.43 ng/ml, and the inter- and intra-assay coefficients of variation (CV) were less than 12.5% and 8.5%, respectively. Plasma IGF-1 was measured by double antibody RIA utilizing NHPP anti-human- IGF-1 (AFP4892898), standard hIGF-1 (Amersham, lot # 30), and labeled ^{125}I-IGF-1 (Amersham, code IM172). Before the RIA, plasma samples were extracted according to the method of Daughaday et al. (1980). Sensitivity of the IGF-1 assay was 0.82 ng/ml, and the CV of inter- and intra-assay was 11.3 ng/ml and 6.2 ng/ml, respectively. Plasma T3 and T4 were measured by RIA utilizing RIA-mat-T3 and RIA-mat-T4 kits (Sangtec, USA).

Statistical analysis

Data from the feeding study were subjected to least squares analysis of variance (ANOVA) according to the general linear models procedure of SAS (1985). All data were presented as means±SEM. Differences between treatment groups were analyzed by Duncan's multiple range test (Duncan, 1955). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Milk yield, milk composition, and DMI

Daily DMI, and the parameters of milk quality such as fat, protein, lactose, and SNF were similar across the treatments (Table 4). The present results were similar to

Table 4. Changes of milk yield and content in cows fed experimental diets

Item	Control	7.5% ESB-HTMT	15% ESB-HTMT
DMI (kg/d)[1]	22.2±2.23	21.67±2.45	21.5±2.64
Milk yield (kg/d)	27.8±4.11	26.5±5.36	28.5±3.27
Milk fat (%)	3.88±0.231	3.91±0.198	3.77±0.214
Milk protein (%)	3.08±0.121	3.19±0.145	3.09±0.139
Lactose (%)	4.77±0.111	4.57±0.123	4.62±0.123
Solid non fat (%)	8.56±0.356	8.51±0.467	8.40±0.333

Data is the average of 7 heads±SD. [1] Dry matter intake.

those of others who reported that high oil supplementation did not affect dairy characteristics and DMI (Spahr et al., 1975; Atwell et al., 1988; LaCount et al., 1995; Chouinard et al., 1998; Dhiman et al., 1999).

An increase in dietary fat content of 1.4-1.7% was probably not sufficient to change milk yield, milk composition and DMI. However, Block et al. (1981) reported that heating of oil produced reducing agents that were capable of capturing hydrogen ions and possibly inhibited methanogenesis in the rumen. Reduced methanogenesis spares other hydrogen ions for the production of propionate, which could lead to milk fat depression. Our heating time of 0.3 seconds was very short and extrusion temperatures within the range of 125°C to 170°C were lower than the temperatures used in previous studies that had reported milk fat depression, e.g., 155°C (Block et al., 1981) and 149°C (Guillaume et al., 1991). In our previous study, as the diets containing ESB were treated by HTMT processing, 40.4% of dietary fat (2.12% out of 5.23% total fat) was found in bound form, quantified by determining the difference between the acid hydrolysis method and the ether extract method for the fat analysis. By comparison, in the non-HTMT diet, there was 30.4% dietary fat (1.32% out of 4.39% total fat) in bound form (data not shown). Therefore, the HTMT treatment used in our experiment might have prevented the formation of such reducing agents responsible for the decrease in milk fat and yields.

Fatty acid composition of milk

Supplementation of HTMT treated diets containing 7.5% or 15% of ESB significantly increased the content of milk cis-9, trans-11 CLA content (i.e., 0.27, 0.55, 0.56 from control, 7.5% ESB, and 15% ESB supplemented diet, respectively) and tended to increase the *trans*-vaccenic acid formation (Table 5). The cis-9, trans-11 -CLA was initially identified by Kepler et al. (1966) as being an intermediate agent in the bio-hydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrisolvens*. Increases in CLA content in milk (Kelly et al., 1998; Mir et al., 1999) have been observed when oil sources which were high in C18:2 were supplemented. It was also indicated that an increase in linoleic acid intake was one of the feeding strategies to enrich CLA in ruminant fats since linoleic acid is the main precursor of CLA (Bessa et al., 2000; Kim et al., 2003). However, the CLA was an intermediate product in the bio-hydrogenation pathway, so the initial isomerization is followed by the saturation of the cis-9 double bond rapidly through the reductase resulting in trans-vaccenic acid. Kim et al. (2000) and Wang et al. (2002; 2003) suggested that rumen bacteria produced greater amounts of cis-9, trans11-CLA *in vitro* only when the concentration of linoleic acid was high enough to inhibit bacterial growth. In addition the endogenous synthesis of cis-9, trans-11 CLA from trans-vaccenic acid (trans-11 C18:1) in the mammary gland has been proposed as being the major pathway of CLA synthesis in the ruminants (Griinari et al., 2000).

In the present study, supplementation of ESB at two levels resulted only in approximately a 1.5-percentage

Table 5. Changes of milk fatty acid composition in cows fed experimental diets

Fatty acids	Control	7.5% ESB-HTMT	15% ESB-HTMT
C8:0	0.41±0.08^{b}	0.60±0.05^{ab}	0.69±0.05^{a}
C10:0	1.52±0.17^{b}	1.84±0.16^{ab}	2.17±0.16^{a}
C12:0	2.48±0.16	2.01±0.54	2.73±0.47
C14:0	9.69±0.73^{ab}	8.69±0.85^{b}	11.35±0.25^{a}
C14:1	0.78±0.12	0.80±0.10	0.73±0.06
C15:0	0.92±0.03^{b}	0.88±0.04^{b}	1.04±0.05^{a}
C16:0	29.37±1.10	28.73±0.83	28.38±1.09
C16:1	0.23±0.05	0.1±0.03	0.34±0.11
C17:0	0.82±0.05	0.66±0.1	0.63±0.08
C18:0	14.83±1.76	15.36±1.31	15.41±1.23
C18:1(trans-11)	4.35±0.49	5.28±0.36	4.50±0.39
C18:1 (cis-9)	28.34±1.39^{ab}	30.00±0.91^{a}	25.59±1.35^{b}
C18:2 (cis- 9,cis-12)	1.67±0.08	1.52±0.03	1.56±0.05
CLA (cis-9,trans-11)	0.27±0.08^{b}	0.55±0.05^{a}	0.56±0.08^{a}
C18:3	0.39±0.12	0.26±0.02	0.32±0.04
C20:1	0.31±0.19	0.18±0.04	0.17±0.04
C22:0	0.21±0.05	0.25±0.05	0.16±0.06

$^{a, b, c}$ Means in the same row with different superscripts differ.

increase in total lipid in the experimental diets (Table 2). The total lipid content was 4.31 and 4.56% for treatment 1 and 2, respectively. The amount of lipid supplementation was far lower than that of previous studies, e.g., 7% (Bell and Kennelly, 2000) and 6% (Ivan et al., 2001). Thus, we may hypothesize that the increased CLA proportion in our experiment was caused mainly by HTMT processing of concentrate containing ESB supplementation. In the HTMT treatment of the experimental diets, the free lipid fraction could be converted to bound-fat with starch. In addition, the heat treatment of ESB produces low degradable protein through the Maillard reaction (Ljøkjel et al., 2000). Therefore, it may slowly release the lipid, and cause an increase in CLA production in the rumen without a negative effect of dietary fat on ruminal fermentation. Additionally, the relatively high concentration of trans-vaccenic acid in milk fat could be responsible for the decrease in the proportion of short- and medium-chain fatty acid. As expected, milk fat was not significantly altered across the treatment possibly due to similar trans-C18:1 fatty acid content. It is fascinating that CLA supplements in our trials did not induce milk fat depression, since it is well established that trans-C18:1 fatty acid can inhibit fatty acid synthesis in mammary tissue (Gaynor et al., 1994).

Metabolites and hormones

Supplementation of HTMT-treated diets containing 7.5% or 15% of ESB significantly decreased the concentration of plasma insulin and glucose at $p<0.05$ (Tables 6 and 7). In contrast, plasma TG, NEFA and HDL-cholesterol were increased in 7.5% and 15% ESB treatment groups (Table 7). However, no difference was observed in plasma LDL-cholesterol, IGF-1, T3, T4, and leptin concentrations across treatments.

The metabolic effects in blood of dairy cows seemed to be associated with dietary fat supplementation. In this study, the concentration of plasma insulin and glucose was decreased by 7.5% ESB and 15% ESB, respectively. The intake of polyunsaturated plant oils has been reported to decrease basal serum insulin concentration in dairy cows fed at the same energy level (Grum et al., 1996). Gaynor et al. (1995) reported that the percentage of glucose and insulin typically increased as the percentage of concentrate in the diet of lactating dairy cows was increased. Increased responses in the plasma lipid fraction (NEFA, TG, HDL-cholesterol) with dietary unsaturated oils were consistent with previous results (Grum et al., 1996; Loor et al., 2002, 2005). The increased NEFA and TG may result from incomplete uptake of NEFA by peripheral tissues after hydrolysis of triglyceride in chylomicrons or very low-density lipoproteins by lipoprotein lipase (Grummer et al., 1991; Chilliard, 1993). The increased concentration of HDL-C in plasma may have been due to the increase in dietary USFA.

IGF-1 and GH are closely related to the regulation of mammalian epithelial cell proliferation and differentiation (Plath-Gabler et al., 2001). IGF-1, which is derived mainly from the liver and GH-dependent (Breier et al., 1988; Bass et al., 1991), acts as a mediator of mammogenesis and regulates the yield and components of milk in mammary epithelial cells (Breier and Sauerwein, 1995; Plath-Gabler et al., 2001). However, in the present study, the IGF-1 levels were not affected by ESB supplementation, although the concentration of plasma GH increased in ESB groups (Table 7). Moreover, an increased GH did not affect the yield of milk and its components (Table 4). The endogenous

Table 6. Changes in plasma metabolites in cows fed experimental diets

Item	Control	7.5% ESB-HTMT	15% ESB-HTMT
Glucose (mg/dl)	83.3±2.03[a]	68.8±2.50[b]	61.3±2.35[b]
NEFA (mEq/L)	4.2±0.52[b]	6.2±1.66[a]	7.3±1.97[a]
TG (μM/L)	34.3±1.61[b]	55.9±1.11[a]	61.3±1.21[a]
HDL-Chol (mg/dl)	132.3±2.34[b]	152.5±3.28[ab]	177.0±2.55[a]
LDL-Chol (mg/dl)	27.4±1.56	26.2±2.12	25.6±2.54

[a, b] Means in the same row with different superscripts differ.

Table 7. Changes in plasma metabolic hormones in cows fed experimental diets

Item	Control	7.5% ESB-HTMT	15% ESB-HTMT
Insulin (μIU/ml)	37.4±1.48[a]	26.2±1.57[b]	22.5±1.22[b]
GH (ng/ml)	4.3±0.56[b]	6.2±0.44[a]	6.0±0.21[a]
IGF-1 (ng/ml)	130.2±6.94	121.95±5.98	132.6±8.05
Leptin (ng/ ml)	4.3±0.12	4.2±0.44	5.0±0.81
T3 (mg/dl)	168.0±5.52	171.1±12.66	158.3±9.97
T4 (μg/dl)	5.5±0.12	5.4±0.35	6.0±0.25

[a, b] Means in the same row with different superscripts differ.

GH/IGF-1 axis is affected by energy and protein supply in steers (Ronge et al., 1988; Lee et al., 2000, 2005). Furthermore, the rate of production of IGF-1 was shown to be influenced by the concentration and affinity of hepatic GH receptors in steers (Breier et al., 1988) and sheep (Bass et al., 1991). The number of hepatic binding sites for bovine GH were greatly affected by nutrition levels in hepatic tissues of cows during mid-lactation (Newbold et al., 1997). Indeed, a significant correlation was found between plasma concentrations of IGF-1 and the capacity of the high-affinity somatotrophic binding sites (Breier et al., 1988). In addition, thyroid hormones regulate liver IGF-1 production and previous *in vitro* and *in vivo* studies showed that thyroid hormones potentiate hepatic IGF-1 synthesis in response to GH (Tollet et al., 1990; Wolf et al., 1989). On the other hand, Grum et al. (1996) reported that the concentrations of T3 and T4 tended to increase when fat was fed in dairy cow. However, we did not observe similar trends in our experiment (Table 7). Besides, plasma leptin levels depend upon the energy uptake in cattle (Tsuchiya et al., 1998; Amstalden et al., 2000), and leptin regulates GH secretion in sheep (Roh et al., 1998). However the present results did not show a significant difference among these treatments.

CONCLUSION

In conclusion, the CLA content in milk could be enhanced by dietary HTMT treatment without any adverse effects on milk yield and composition. In addition, the unchanged milk fat composition and milk yield may be associated with a constant level of IGF-1 and thyroid hormone responses to increased endogenous GH by the HTMT-treated diets, because these hormones act as mediators of mammogenesis, and regulate the yield of milk and its components in mammary epithelial cells.

REFERENCES

AOAC. 1984. Official methods of analysis (14th ed.). Association of Official Analytical Chemists, Washington, DC.

AOAC. 1990. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC. Index No. 973.18.

Amstalden, M., M. R. Garcia, S. W. Williams, R. L. Stanko, S. E. Nizielski, C. D. Morrison, D. H. Keisler and G. L. Williams. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prebubertal heifers: relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63:127-133.

Atwell, D. G., E. H. Jaster, K. J. Moore and R. L. Fernando. 1988. Evaluation of high oil and corn silage for lactating dairy cows. J. Dairy Sci. 71:2689-2698.

Bass, J. J., J. M. Oldham, S. C. Hodgkinson, P. J. Fowke, H. Sauerwein, P. Molan, B. H. Breier and P. D. Gluckman. 1991. Influence of nutrition and bovine growth hormone (GH) on hepatic GH binding, insulin-like growth factor-I and growth of lambs. J. Endocrinol. 128:181-186.

Bateman, H. G. and T. C. Jenkins. 1998. Influence of soybean oil in high fiber diets fed to nonlactating cows on ruminal unsaturated fatty acids and nutrient digestibility. J. Dairy Sci. 81:2451-2458.

Bell, J. A. and J. J. Kennelly. 2000. Producing conjugated linoleic acid enriched milk through practical dairy nutrition. Conference abstract: 8th World Congress on Clinical Nutrition, Bankok, Thailaid. December. pp. 17-20.

Belury, M. A. 1995. Conjugated dienoic linoleate, a polyunsaturated fatty acid with unique chemoprotective properties. Nutr. Rev. 53:83-89.

Bessa, R. J., J. Santos-Silva, J. M. Ribeiro and A. V. Portugal. 2000. Reticulo-rumen biohydrogenation and the enrichment of ruminat edible products with linoleic acid conjugated isomers. Appl. Environ. Microbiol. 63:201-211.

Block, E. L., D. Muller, L. C. Griel and D. L. Garwood. 1981. Brown midrib-3 corn silage and heated extruded soybeans for early lactating dairy cows. J. Dairy Sci. 64:1813-1819.

Breier, B. H. and H. Sauerwein. 1995. Regulation of growth in ruminants by the somatotropic axis. In: Ruminant physiology: Digenstion, metabolism, growth and reproduction (Ed. W. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke). Stuttgart: Enke Verlag. pp. 102-123.

Breier, B. H., P. D. Gluckman and J. J. Bass. 1988. The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high- and low-affinity somatotrophic biding sites. J. Endodrinol. 116:169-177.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. J. Dairy Sci. 76(12): 3897-3931.

Chouinard, P. Y., V. Girard and G. J. Brisson. 1998. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. J. Dairy Sci. 81:471-481.

Chouinard, P. Y., L. Corneau, W. R. Butler, Y. Chilliard, J. K. Drackley and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84:680-690.

Daughaday, W. H., I. K. Mariz and S. L. Blethen. 1980. Inhibition of assess of bound somatomedin to membrane receptor and immunobinding sites: A comparision of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol extraction serum. J. Clin. Endocrinol. Metab. 51:781-788.

Dhiman, T. R., G. R. Anand, L. D. Satter and M. W. Pariza. 1999. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82:2146-2156.

Duncan, D. B. 1955. Multiple ranges and multiple F test. Biometrics 11:1-42.

Duckett, S. K., J. G. Andrae and F. N. Owens. 2002. Effect of high oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80:3353-3360.

Folch, J., M. Lee and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:497-509.

Gaynor, P. J., D. R. Waldo, A. V. Capuco, R. A. Erdman, L. W. Douglass and B. B. Teter. 1995. Milk fat depression, the glucogenic theory, and trans-C18:1 fatty acids. J. Dairy Sci. 78:2008-2015.

Gaynor, P. J., R. A. Erdman, B. B. Teter, J. Sampugna, A. V. Capuco, D. R. Waldo and M. Hamosh. 1994. Milk fat yield and composition during abomasal infusion of cis or trans octadecenoates in Holstein cows. J. Dairy Sci. 77:157.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. Nurmela and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J. Nutr. 130:2285-2291.

Griinari, J. M., D. A. Dwyer, M. A. McGuire and D. E. Bauman. 1996. Partially hydrogenated fatty acids and milk fat depression. J. Dairy Sci. 79(Suppl.1):177(abstr).

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Grummer, R. R. and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838-3852.

Guillaume, B., D. E. Ottwerby, M. D. Stern, J. G. Linn and D. G. Johnson. 1991. Raw or extruded soybeans and rumen-protected methionine and lysine in alfalfa-based diets for dairy cows. J. Dairy Sci. 74:1912-1917.

Harfoot, C. G. and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. In: The rumen microbial ecosystem (Ed. P. N. Hobson). Elsevier Appl. Sci., London and NY. pp. 285-322.

Houseknecht, K. L., J. P. Vanden Heuvel, S. Y. Moya-Camarena, C. P. Portocarrero, L. W. Peck, K. P. Nickel and M. A. Belury. 1998. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem. Biophys. Res. Commun. 244:678-682.

Ivan, M., P. S. Mir, K. M. Koenig, L. M. Rode, L. Neill, T. Entz and Z. Mir. 2001. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Rumin. Res. 41:215-227.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76(12):3851-3863.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. V. Amburgh and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128: 881-885.

Kepler, C. R., K. P. Hirons, J. J. McNeill and S. B. Tove. 1966. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. J. Biol. Chem. 241:1350-1354.

Kim, Y. J., R. H. Liu, D. R. Bond and J. B. Russell. 2000. Effect of linoleic acid concentration on conjugated linoleic acid production by *Butyrivibrio fibrisolvens* A38. Appl. Environ. Microbiol. 66:5226-5230.

Kim, Y. J., K. W. Lee and H. J. Lee. 2003. Increase of conjugated Linoleic acid level in milk fat by bovine feeding regimen and ure fractionation. J. Microbiol. Biotechnol. 13:22-28.

LaCount, D. W., J. K. Drackley, T. M. Cicela and J. H. Clark. 1995. High oil corn as silage or grain for dairy cows during an entire lactation. J. Dairy Sci. 78:1745-1754.

Lee, H. G., R. A. Vega, L. T. Phung, N. Matsunaga, H. Kuwayama and H. Hidari. 2000. The effect of growth hormone releasing peptide-2 (KP102) administration on plasma insulin-like growth factor (IGF)-I and IGF-binding proteins in Holstein steers on different planes of nutrition. Domest. Anim. Endocrinol. 18(3):293-308.

Lee, H. G., Y. J. Choi, S. R. Lee, H. Kuwayama, H. Hidari and S. K. You. 2005. Effects of dietary protein and growth hormone-releasing peptide (GHRP-2) on plasma IGF-1 and IGFBPs in Holstein steers. Domest. Anim. Endocrinol. 28:134-146.

Lee, H. S., E. J. Kim, J. S. Cho, H. J. Cho, S. K. Lee, K. C. Jung and J. H. Y. Park. 1994. Conjugated linoleic acid decreases phosphorylated Akt levels in Mat-LyLu (MLL) rat prostate cancer cells. Food Sci. Biotechnol. 13:353-357.

Ljokjel, K., O. M. Harstad and A. Skrede. 2000. Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. Anim. Feed Sci. Technol. 84:83-95.

Loor, J. J., A. Ferlay, A. Ollier, M. Doreau and Y. Chilliard. 2005. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J. Dairy Sci. 88:726-740.

Loor, J. J., J. H. Herbein and C. E. Polan. 2002. Trans18:1 and 18:2 isomers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. J. Dairy Sci. 85:1197-1207.

Mir, Z., L. A. Goonewardene, E. Okine, S. Jaegar and H. D. Scheer. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and fatty acid profiles in goats milk. Small Rumin. Res. 33:137-143.

Murphy, J. J., J. F. Connolly and G. P. McNeill. 1995. Effects on cow performance and milk fat composition of feeding full fat soybeans and rapeseeds to dairy cows at pasture. Livest. Prod. Sci. 44:13-25.

National Research Council. 1989. Nutrient requirement of dairy cattle. 6th rev. ed. Natl. Acad. Sci. Washington, DC.

Newbold, J. A., R. B. Heap, C. G. Prosser, R. H. Phipps, F. Adriaens and D. L. Hard. 1997. The effect of bovine somatotropin and diet on somatotropin binding sites in hepatic tissue of lactating dairy cows. J. Dairy Sci. 80:1085-1091.

Nicolosi, R. J., E. J. Rogers, D. Kritchevski, J. A. Scimeca and P. J. Huth. 1997. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherogenesis in hypercholesterolemic hamsters. Artery 22:266-277.

Palmquist, D. L. 1988. The feeding value of fats. In: Feed Science: World Animal Science. Disciplinary Approach B4. Elsevier, Amsterdam. (Ed. E. R. Orskov). pp. 293-311.

Park, Y., K. J. Albright, W. Liu, J. M. Storkson, M. E. Cook and M. W. Pariza. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids 32:853-858.

Plath-Gabler, A., C. Gabler, F. Sinowatz, B. Berisha and D. Schams. 2001. The expression of the IGF family and GH receptor in the bovine mammary gland. J. Endocrinol. 168:39-48.

Lepage, G. and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. J. Lipid Res. 27:114-120.

Roh, S., I. J. Clarke, R. Xu, J. W. Goding, K. Loneragan and C. Chen. 1998. The *in vitro* effect of leptin on basal and growth hormone-releasing hormone-stimulated growth hormone secretion from the ovine pituitary gland. Neuroendocrinology 68:361-364.

SAS. 1985. SAS User's guide: Statistical Analysis Systems Institute, Inc., Cary, NC.

Spahr, S. L., J. H. Byers and J. H. Clark. 1975. No advantage for high oil corn silage when feeding dairy cows. Illinois Res. 17:16-17.

Tollet, P., B. Enberg and A. Mode. 1990. Growth hormone (GH) regulation of cytochrome P-450IIC12, Insulin-like growth factor-I (IGF-I), and GH receptor messenger RNA expression in primary rat hepatocytes: a hormonal interplay with insulin, IGF-1 and thyroid hormone. Mol. Endocrinol. 4:1934-1942.

Tsuchiya, T., Y. Nagao, A. Ozawa, M. Matsumoto, K. Sugahara, T. Kubo and H. Kato. 1998. Decrease of the obese gene expression in bovine subcutaneous adipose tissue by fasting. Biosci. Biotechnol. Biochem. 62:2068-2069.

Van Dijk, H. J., G. D. O'Dell, P. R. Perry and L. W. Grimes. 1983. Extruded versus raw ground soybeans for dairy cow in early lactation. J. Dairy Sci. 66:2521-2533.

Wang, J. H. and M. K. Song. 2001. Effect of sources and levels of carbohydrates on fermentation characteristics and hydrogenation of linoleic acid by rumen bacteria *in vitro*. Asian-Aust. J. Anim. Sci. 14:48-53.

Wang, J. H. and M. K. Song. 2003. PH affects the *in vitro* formation of *cis*-9, *trans*-11CLA and *trans*11-octadecenoic acid by ruminal bacteria when incubated with oilseeds. Asian-Aust. J. Anim. Sci. 16:1743-1748.

Wang, J. H., M. K. Song, Y. S. Son and M. B. Chang. 2002. Addition effect of seed-associated or free linseed oil on the formation of *cis*-9, *trans*-11 conjugated linoleic acid and octadecenoic acid by ruminal bacteria *in vitro*. Asian-Aust. J. Anim. Sci. 15:1115-1120.

Wolf, M., S. H. Ingbar and A. C. Moses. 1989. Thyroid hormone and growth hormone interact to regulate insulin-like growth factor-1 messenger ribonucleic acid and circulation levels in the rat. Endocrinology 125:2905-2914.