Slovak J. Anim. Sci., 44, 2011 (2): 52-58

ISSN 1337-9984

EFFECT OF DIETARY FAT SUPPLEMENTATION ON MILK COMPONENTS AND BLOOD PARAMETERS OF EARLY-LACTATING COWS UNDER HEAT STRESS

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ABSTRACT

The objective of the present experiment was to evaluate the effect of dietary fat supplementation on milk components and blood parameters of early-lactating cows under heat stress. Cows in group 1 were fed basal diet without dietary fat (T1) supplementation (control). The other four experimental groups were fed 1.5% palmitic acid (T2), rapeseed oil (T3) or soybean oil (T4), and 1.8% fat powder (T5), respectively. The results showed that the average Temperature Humidity Index (THI) was 76.68. The Dry Matter Intake (DMI) was not affected by fat supplementation, but the milk yield increased for cows fed supplemental fat. Milk fat increased significantly by fat supplementation ($P<0.05$), while milk protein and lactose were not significantly altered by fat supplementation ($P>0.05$). The concentration of plasma glucose was increased by fed supplemental fat ($P<0.05$) while that of plasma urea nitrogen was decreased by fed supplemental fat ($P<0.05$). Furthermore, there were significant increases of total triglycerides, total cholesterol and high density lipoprotein (HDL) ($P<0.05$), but low-density lipoprotein (LDL) did not differ significantly by fat supplementation ($P>0.05$). These results indicate that supplementation of dietary fat on early-lactating cows during hot weather can alter milk components and blood parameters, which may be beneficial for enhancement of energy balance and alleviation of heat stress.

Key words: heat stress; dietary fat supplementation; dairy cows; milk components; blood parameters

INTRODUCTION

Livestock undergo metabolic changes during heat stress (Okab, 2008; Olexiková, 2007), which may lead to decreases in Dry Matter Intake (DMI) and subsequent declines in performance (Brouček *et al.*, 2009). In an attempt to minimize the negative effects of these changes and maintain high lactation performance, it usually increases dietary energy concentration and reduces heat stress for dairy cows by supplemental fat to dairy diets during hot weather (Gaugha *et al.*, 2008; Warntjes *et al.*, 2008; Wang *et al.*, 2009). Lipids can trigger a gut-brain-liver axis to regulate energy metabolism, which is beneficial to keep energy balance of dairy cows under heat stress (Harvatine *et al*., 2006). Less heat may be produced in the rumen during digestion of fat-supplemented diets as fatty acids are not digested in the rumen. Studies showed that dietary fat supplementation can improve production performance of dairy cows (Warntjes *et al.*, 2008; Santos *et al.*, 2009).

In today's milk market, where fat and protein are the major components of milk, dietary supplementation of fat can lead to changes in the composition of milk. Juchema et al. (2008) observed that milk fat was not affected by fat source, while dietary fat decreased the milk protein content. José et al. (2010) reported that milk fat increased significantly by supplemental whole raw soybean for lactating dairy cows, and there was no effect

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Received: January 21, 2011
Accepted: April 7, 2011

of fat sources added to the diet on milk protein.

However, little information is available about the effect of fat supplementation on milk production, milk components and blood parameters of early-lactating cows under heat stress. The objectives of this study were to determine the effect of fat supplementation on milk components and blood parameters of early-lactating cows under summer heat, by adding a rumen fat to the concentrate.

MATERIAL AND METHODS

Experimental Design

Thirty Chinese Holstein cows weighing 500 kg ± 50 kg, 2-4 parity and days in milk (DMI, 15-24 d) were randomly assigned to 5 dietary treatment groups with 6 cows per group. Cows in group 1 were fed a basal diet without dietary fat (T1) supplementation (control). The other four experimental groups were fed 1.5% palmitic acid (T2), rapeseed oil (T3) or soybean oil (T4), and 1.8% fat powder (T5), respectively.

Experimental Diets

The nutrient levels of diets were formulated to meet the recommendations by NRC (2001) and maintenance requirements were increased by 20% because of hot weather in all the groups. Experimental diets were isoenergetic and isonitrogenous with forage-to-concentrate of 1:1 (DM). The forages consisted of alfalfa meal, rice straw, brewer grain and grass. The ingredients and chemical composition of diets are presented in Table 1.

Table 1: Ingredients and chemical composition of diets (DM basis %)

Items	Groups				
	T1	T2	T3	T4	T5
Ingredients					
Corn grain (%)	28.5	27	27	27	26.7
Wheat bran (%)	7.5	7.5	7.5	7.5	7.5
Rapeseed meal (%)	4.5	4.5	4.5	4.5	4.5
Soybean meal (%)	2.5	2.5	2.5	2.5	2.5
Cottonseed meal (%)	4.5	4.5	4.5	4.5	4.5
Palmitic acid (%)	-	1.5	-	-	-
Rapeseed oil (%)	-	-	1.5	-	-
Soybean oil (%)	-	-	-	1.5	-
Fat powder (%)[a]	-	-	-	-	1.8
Limestone (%)	1.0	1.0	1.0	1.0	1.0
Salt (%)	0.5	0.5	0.5	0.5	0.5
Sodium bicarbonate (%)	0.5	0.5	0.5	0.5	0.5
Premix[b] (%)	0.5	0.5	0.5	0.5	0.5
Grass (%)	17	17	17	17	17
Alfalfa meal (%)	15	15	15	15	15
Brewer grain (%)	7	7	7	7	7
Rice straw (%)	11	11	11	11	11
Nutrient level					
NEL (MJ/Kg)[c]	6.12	6.33	6.33	6.33	6.29
CP (%)	16.29	16.15	16.17	16.16	16.12
NDF (%)	37.51	37.35	37.34	37.33	37.32
ADF (%)	23.24	23.18	23.21	23.19	23.16
Ca (%)[c]	0.92	0.92	0.92	0.92	0.92
P (%)[c]	0.45	0.45	0.45	0.45	0.44

[a]. Fat powder: calcium salt of palm oils (Crude fat≥84%, Ca≥6.5%).
[b]. Trace element and vitamin per kg of Premix: VA, 1000 KIU; VD3, 250 KIU; VE, 5000mg; VB5, 10000 mg; Biotin, 60mg; Cu, 1600 mg; Fe, 2500 mg; Zn, 8000 mg; Mn, 2500 mg; I, 100 mg; Se, 60 mg; Co, 20 mg.
[c]. NEL, Ca and P are calculated values. Other nutrient levels are measured values.

Animals and Treatments

The experiment was carried out for 7 weeks followed by 1 week of adjustment in a dairy farm from August 5 to October 29 in 2010. With an increment of 50 g/d the dietary fat attained the expected amount during the adjustment period of the experiment. During the measurement period, the amount of supplemental fat was adjusted according to DMI of cows in the last week to keep supplementation at 1.5% palmitic acid (T2), rapeseed oil (T3) or soybean oil (T4), and 1.8% fat powder (T5), respectively. Cows were fed individually at 05:00, 11:30 and 18:00 h.

Meteorological data collection

The ambient temperature and humidity were recorded daily (at 07:00, 14:00, and 22:00 h) from 1.5 m above floor during experimental period from 2 ends and middle of the barn. Estimates of temperature-humidity index (THI) in the barn were generated using the following formulae: THI = 0.72 (Td+Tw) + 40.6, where td is the dry-building temperature (in degrees of Fahrenheit) and Tw is the relative humidity (Maust *et al*., 1972).

Sampling and Analysis

Body temperature and respiration rate were measured weekly. Body temperature was measured by using a rectal thermometer. Respiration rate was measured by counting flank movements during three uninterrupted, 30-s intervals.

Milk yield was recorded every two weeks from individual cows by weighing the milk. A 50 ml milk sample from individual cows was collected on the last day of each month (at 05:30, 12:30 and 19:30 h) in order to analyze milk components. Milk samples were preserved with potassium dichromate and analyzed for fat, protein and total solids by using MilkoScan FT120.

Blood was collected into heparin-free tubes and centrifugated to obtain plasma at the end of experiment. Plasma was stored at –20°C for further analyses. Plasma samples were later analyzed for total triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, plasma urea nitrogen and plasma glucose levels using automatic biochemical analyzer.

Basal dietary Dry Matter (DM) and Crude Protein (CP) were determined according to AOAC (1990). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined after Van Soest *et al.* (1991).

Statistical analyses

Statistical analysis was performed using SPSS17.0. Data was analyzed by one-way ANOVA. The results were expressed as the means ± standard deviation. Duncan multiple comparisons were used to test the differences between treatments, which were denoted by differential letter superscripts. Significant and extreme differences were set at $P<0.05$ and $P<0.01$, respectively.

RESULTS

Effect of supplementation of dietary fat sources on the body temperature and respiration rate is shown in Table 2. Addition of palmitic acid, soybean oil, rapeseed oil or fat powder was found to decrease the body temperature by 0.2°C and respiratory rate by 1, 2, 1, 1 and 2 beats / min, respectively.

Table 2: Effect of supplementation of dietary fat sources on the body temperature and respiration rate (average)

Items	Groups				
	T1	T2	T3	T4	T5
Respiratory rate (beats/min)	65±1.97	64±2.10	63±2.31	63±2.67	64±1.63
Rectal temperature (°C)	38.7±0.10	38.5±0.14	38.5±0.14	38.5±0.19	38.5±0.18

Table 3: Effect of supplementation of dietary fat sources on dry matter intakes (DMI) of dairy cows (average)

Items	Groups				
	T1	T2	T3	T4	T5
DMI (kg)	15.21±0.23	15.16±0.19	15.21±0.09	15.20±0.22	15.17±0.19

The results showed that the DMI was not affected by fat supplementation (Table 3). Effect of dietary fat supplementation on the milk yield of dairy cows is shown in Table 4. In the control group there was 1.42 kg reduction of milk yield, the decrease being 6.7%. In soybean oil group (T2) milk yield decrease was by 0.42 kg, which is by 2.02%. On the other hand, in fat powder group (T3) an increase by 0.36 kg was noted, the increment accounting for 1.85% in palmitic acid group (T4) there was an increase in milk production by 2.50 kg, the increase being 13.3%; and, in rapeseed oil group (T5) the increased in milk yield was by 0.08 kg, accounting for 0.41% enhancement.

Effect of supplementation of dietary fat sources on the milk components of dairy cows is shown in Table 5. Milk fat significantly increased by supplemental fat ($P<0.05$), while there was no significant difference ($P>0.05$) between milk protein and lactose levels after dietary fat supplementation.

Effect of supplementation of dietary fat on the plasma urea nitrogen and blood glucose levels are shown in Figure 2. The concentration of plasma glucose increased by supplemental dietary fat ($P<0.05$), while the concentration of plasma urea nitrogen decreased by supplemental dietary fat ($P<0.05$).

Table 4: Effect of supplementation of dietary fat sources on the milk yield of dairy cows

Mik yield (kg/d)	Groups				
	T1	T2	T3	T4	T5
Initial (kg)	21.08±4.47	18.75±3.00	19.50±3.16	20.83±2.66	19.42±1.24
Average (kg)	19.67±4.34	21.25±2.82	19.58±3.14	20.21±3.64	20.21±2.76
Change (kg)	-1.42	+2.5	+0.08	-0.42	+0.36

„-“ represents decrease; „+“ represents increase

Table 5: Effect of supplementation of dietary fat sources on the milk components of dairy cows (average)

Items	Groups				
	T1	T2	T3	T4	T5
Milk fat (kg)	3.08±0.23^{a}	3.40±0.26^{b}	3.45±0.25^{b}	3.50±0.36^{b}	3.36±0.16^{ab}
Milk protein (kg)	2.80±0.16	2.84±0.15	2.91±0.17	2.96±0.20	2.83±0.14
Milk solids (kg)	9.91±1.40	9.31±0.66	9.34±0.94	9.94±1.17	9.00±0.49

In the same row, values with different superscripts indicate significant differences ($P<0.05$)

DISCUSSION

The heat stress environment is formed when the ambient temperature and humidity index (THI) exceeds 72, and when THI reaches 76 severe heat stress results. During the experiment, average THI of barn was 76.68, which indicated that the cows were suffering from heat stress (Yu *et al*., 2006; Brouček *et al*., 2009).

Above 26°C, dramatic losses in production can occur although the humidity index will alter the upper critical temperature (Berman *et al*., 1985). In an attempt to dissipate additional body heat, the cow increases her respiration rate, increases sweating rate, increases blood flow to skin, and decreases energy intake (Blackshaw and Blackshaw, 1994). In this study, supplemental fat source decreased the rectal temperature and respiratory rate. Fat has a low heat increment associated with feeding, and is also accompanied by a high energy density. Thus it is utilized with high efficiency, and is an ideal feed additive under heat stress.

Feeding fat diet (3 to 5%) can increase energy intake without toxic effects to ruminal microflora (Palmquist and Jenkins, 1980). In the present study, only 1.5% dietary fat was supplemented on total DM, so that the DMI was not affected by fat supplements.

In this study, milk yield increased by supplemental fat, which is in agreement with previous studies. Warntjes *et al*. (2008) observed that milk yield increased by 1.35 kg/d after supplementation of 450 g/d palmitic acid in lactating dairy cows under heat stress. Santos *et al*. (2009) evaluated the inclusion of 3.30% soybean oil, on total DM basis, in the diet of dairy cows in the transition

Values with different small letter superscripts mean significant difference (P<0.05)

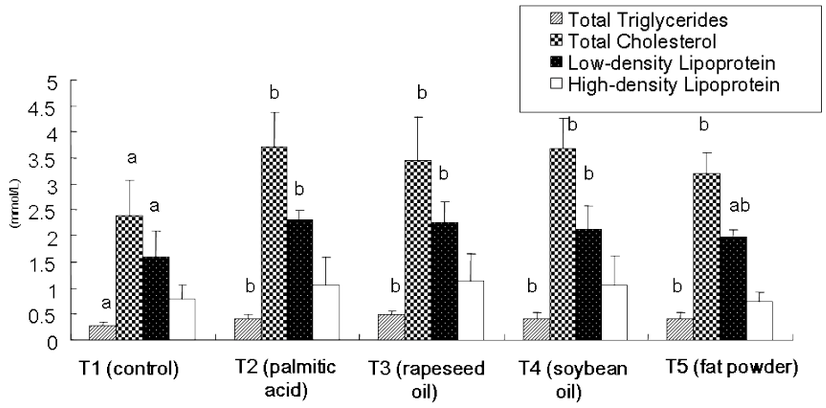

Figure 1: **Effect of supplementation of dietary fat on the serum total triglycerides, total cholesterol, low-density lipoprotein and high-density lipoprotein**

Values with different small letter superscripts mean significant difference (P<0.05)

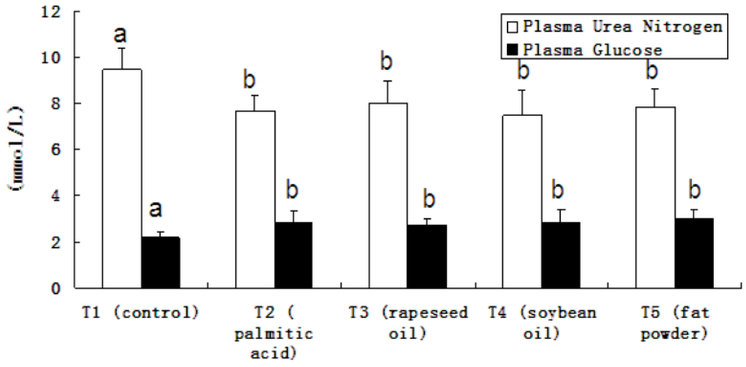

Figure 2: Effect of supplementation of dietary fat on the plasma urea nitrogen and glucose

period and during early lactation. The authors observed that the animals fed soybean oil diet produced 2.53 kg/d more milk than those fed control diet (7.93%). Milk yield was also increased by supplemental fat for early lactation. One reason was that supplemental fat increased dietary energy density, particularly in early lactation when the dairy cow must depend on her body reserves to help satisfy energy requirements for maintenance and lactation (Schroeder, 2004; Yang, 2002).

Warntjes *et al.* (2008) reported that milk fat increased significantly by supplementation of 450 g/d palmitic acid for lactating dairy cows under heat stress. Wang *et al.* (2009) also studied that fat supplement could increase milk fat content under heat stress. In the present study, supplemental fat source could have increased milk fat of dairy cows. The response of milk fat concentration to supplemental fat seems to be dependent upon many factors including the fat concentration and composition in the basal diet and in the supplement as well as the forage source and amount. One possible reason is that supplemental fat increased dietary energy density. Moreover, about 50% of the fat found in milk is synthesized in the mammary gland from acetate and butyrate, while 40 to 45% from the dietary source and less than 10% are derived from the mobilization of adipose tissue (Palmquist and Jenkins, 1980). So, supplemental fat source can increase milk fat of dairy cows.

Milk protein was not significantly affected by treatments, but supplemental fat group could increase milk protein (Table 4). This increase might be due to more ruminal microbes delivering protein for protein synthesis by the mammary gland. When fat is fed, bacteria numbers may increase in concurrence with a decrease in protozoal populations (Sutton *et al*., 1983). The findings of this study are in agreement with previous studies (Warntjes *et al.*, 2008; Perfield *et al*., 2002; Drackley *et al*. 1994).

Barley *et al.* (2009) reported increased concentrations of plasma triglycerides by fat supplementation. Feeding fat to dairy cattle increased plasma cholesterol (Grummer and Carroll, 1991). Thomas *et al.* (1997) indicated that fat-supplemented groups had increased peripheral HDL-cholesterol. In this study, there were significant increases of total triglycerides, total cholesterol and low-density lipoprotein (HDL), but for high-density lipoprotein (LDL) there was no significant difference between fat supplemented animals and control. Plasma triglyceride (TG) mainly derived from the food and the synthesis of the liver, of which 25% have direct involvement in cholesterol (Ch) synthesis. Plasma Ch is mainly associated with LDL or HDL. The transport of Ch in the blood requires plasma lipoproteins, which are LDL and HDL. In the bovine, approximately 5 to 10% of the plasma Ch pool is contributed by LDL, and the remainder is almost exclusively from HDL (Grummer and Carroll, 1991). The synthesis of triglycerides and Ch also must rely on the transport of blood, so blood of TG, Ch, LDL and HDL levels reflected the in vivo metabolism of lipids. Strong lipid metabolism can release more energy to decrease the duration and magnitude of negative energy status.

In this study, concentration of plasma glucose was increased by supplemental dietary fat. This was in agreement with previous report indicating that fat has greater concentrations of glucose in cows (Juchema *et al.*, 2008). One possible reason was that supplemental fat would provide precursors for gluconeogenesis, thereby increasing blood glucose concentration. Another possible reason was that glycolysis of sugar was inhibited by supplemental dietary fat in order to improve their energy levels.

As milk is secreted in the mammary gland, urea diffuses into and out of the mammary gland, equilibrating with urea in the blood. Because of this process, milk urea nitrogen (MUN) is proportional to plasma urea nitrogen (Broderick and Clayton, 1997), and total urinary nitrogen excretion is linearly related to milk urea nitrogen (Jonker *et al.*, 1998). So plasma urea nitrogen is an indicator of diet adequacy and nitrogen utilization efficiency in lactating dairy cattle. Usually the plasma urea nitrogen is relatively stable and is generally influenced by the consumption of nitrogen, as well as by the local secretion of endogenous nitrogen. When the body has inadequate supply of glucose, the cows spend part of the protein synthesis through amino acid gluconeogenesis glucose, leading to increase in plasma urea nitrogen and decrease in nitrogen deposition.

CONCLUSION

The present study suggests that milk yield, milk fat and blood parameters are affected by the fat supplementation in lactating cows. Dietary fat supplementation may be suitable for early-lactating cows under heat stress.

ACKNOWLEDGEMENT

This work was supported by the program for public welfare behaviour (Agriculture) research fund of the Agricultural Department of China (No. nyhyzx07-036-03).

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