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Effect of different concentrations of dietary safflower seed on milk yield and some rumen and blood parameters at the end stage of lactation in dairy cows

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ABSTRACT - In this study, the effects of different concentrations of dietary safflower seeds (SS) were examined for milk production, milk fat and some rumen and blood parameters at the end stage of lactation in dairy cows. Four Holstein cows were assigned to a 4 × 4 Latin Square design with four stages. All stages had 14 d of adaptation and 7 d of data collection periods. The diets were formulated as isoenergetic and isonitrogenous. Cows were fed four concentrate mixtures containing 0% (Control; C), 12.5% (S-I), 25% (S-II), or 37.5% (S-III) crushed SS during the experimental period. Safflower seed intake was distributed as 0 (C), 1 (S-I), 2 (S-II) and 3 (S-III) kg/d/cow. Cows were fed 8 kg concentrate, 2 kg wheat straw, and corn silage *ad libitum* (approximately 20 kg). Diet S-III caused a decrease in efficiency of milk production and diet S-II provided a much further efficiency in milk production (C = 13.39±0.23, S-I = 12.94±0.26, S-II = 13.46±0.24 and S-III = 11.83±0.52 kg). Diets had no significant effect on milk fat (C = 3.99±0.18, S-I = 4.09 ± 0.16, S-II = 3.87±0.35 and S-III = 3.75±0.30%). There was no difference in rumen fluid and blood parameters. Short-time feeding of up to 2 kg/d safflower seed had no negative effects on milk yield, milk fat, and some serum parameters, but 3 kg/d safflower seed reduced milk production. Safflower seed can be safely fed at up to two kilograms daily at the end stage of lactation in dairy cows.

Key Words: milk, oilseed, rumen parameter

Introduction

The Asteraceae family is the largest family of vascular plants and includes many commercially important species like sunflower, safflower, globe artichoke, Jerusalem artichoke and yacon. The safflower plant is 0.6-1.5 m high and produces many branches with heads at the ends. Each head, as in other thistles, consists of numerous flowers; each normally produces a single seed. A head may produce 20-100 small seeds like sunflower seed (FAO, 2004).

The safflower seed (SS) contains about 12-17% crude protein, 25-40% ether extract and 20-33% crude fiber. The nutrient composition of SS changes depending on region, soil and variety. Safflower oil is also rich in polyunsaturated fatty acids (78% linoleic acid), which play an important role on reducing the level of blood cholesterol. Uysal et al. (2006) reported that safflower *(Carthamus tinctorius* L.) had been traditionally grown for vegetable oil production in Isparta, Turkey; the seed oil of most of Safflower lines is rich in linoleic acid and poor in oleic acid in Turkey and the total tocopherol content of safflower oils is between 131.6 and 163.2 mg/100 g. After safflower seed is used for extraction of the oil, its by-product, safflower cake, is used as animal feed.

In safflower seed and seed cake, the methionine and isoleucine contents are hardly adequate, but lysine is quite limited (Nimbkar, 2010). Ivan et al. (2004) reported that the use of sunflower seed supplementation in high-concentrate diets for ruminants reduces the rumen fauna.

Yuk et al. (2002) found that safflower seed extract is effective for the treatment of diseases such as osteoporosis and bone resorption. It is well known that dietary phospholipids lower serum cholesterol levels effectively and crude safflower phospholipids can be used as a feed ingredient to reduce the fatty liver syndrome in laying hens (An et al., 1997). Bell et al. (2006) reported that additional safflower oil at 60 g/kg DM in the feed increased the conjugated linoleic acid (CLA) content in cow milk. The anti-carcinogenic effects of CLA have been widely accepted and the best natural source of CLA is ruminant milk (Dschaak et al., 2010).

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The objective of this experiment was to determine the effects of diets containing 0, 1, 2 and 3 kg/d SS on milk yield, milk fat and some blood and rumen parameters of dairy cows.

Material and Methods

Four Holstein cows with approximately same milk production and body weight, at the end stage of lactation periods on a private farm were used in the experiment. Cows had 13±0.7 kg milk production, 500±18 kg live weight and were at the 272±12 d of 3rd lactation. The animals were housed on a farm and assigned to a 4 × 4 Latin square design with four periods. Each experimental period lasted 21 d (14 d for dietary adjustment and 7 d for data collection).

The diets were formulated as isoenergetic and isonitrogenous (Table 1). Cows were fed four concentrate mixtures containing 0% (Control = C), 12.5% (S-I), 25% (S-II), or 37.5% (S-III) crushed SS during the experimental period. Safflower seed intake was arranged as 0 (C), 1 (S-I), 2 (S-II) and 3 (S-III) kg/d/cow. Cows were fed 8 kg concentrate (4 kg for the morning feeding and 4 kg for the afternoon feeding), 2 kg wheat straw (morning feeding) and corn silage (afternoon feeding) *ad libitum* (approximately 20 kg). No concentrate and straw refusals were observed. The animals had free access to water.

Table 1 - Composition of the concentrate mixtures and nutrients (DM basis)

Ingredients (g/kg)	Diets			
	Control	S-I	S-II	S-III
Barley	350	210	110	50
Wheat	124	166	180	220
Wheat bran	60	60	44	10
Sunflower meal	200	200	200	150
Safflower seed	0	125	250	375
Cottonseed meal	170	144	100	52
Soybean meal	61	60	81	108
Dicalcium phosphate	1	1	1	1
Limestone	25	25	25	25
Salt	5	5	5	5
Mineral mixture[1]	2	2	2	2
Vitamin mixture[2]	2	2	2	2
Analyzed values on DM basis (g/kg)				
Dry matter	887.6	897.3	902.6	904.2
Crude protein	188.2	187.8	186.5	188.9
Ether extract	30.7	64.4	108.6	134.7
Ash	90.1	88.6	82.8	87.6
Acid detergent fiber	155.6	178.2	196.7	215.2
Neutral detergent fiber	352.6	372.9	429.0	442.1
ME[3] (Mj/kg)	9.96	9.92	10.01	9.92

[1] Per kg of mineral premix: Mn - 50,000 mg; Zn - 50,000 mg; Fe - 50,000 mg; Cu - 10,000 mg; I - 800 mg; Co - 150 mg; Se - 150 mg.
[2] Per kg vitamin premix: vitamin A - 15,000,000 IU; vitamin D3 - 3,000,000 IU; vitamin E - 30,000 mg.
[3] Metabolizable energy, calculated from diet software.

Cows were milked twice a day, at 07.00 h and 16.30 h. Milk yield was recorded during data collection period (7 d) in both morning and afternoon milkings. Blood and rumen fluid samples were collected two hours after the morning feeding on the fourth day of each data collection period (7 d). Rumen fluid samples were collected via a stomach tube. Blood samples were collected from the *vena jugularis* with a Vacutainer needle. Serum urea levels (with a commercial kit), serum beta-carotene and vitamin A (Suzuki and Katoh, 1990) and plasma vitamin E levels (Baker and Frank, 1968) were determined using a spectrophotometer (Shimatzu UV1200).

Milk fat levels were determined by the Gerber Method (Marshall, 1992). Feeds were analyzed according to the AOAC (1984), Van Soest (1963) and Crampton and Maynard (1938). Rumen total volatile fatty acids (TVFA) and NH_3-N were measured according to Markham (1942). Rumen fluid pH was measured using a pH meter (Metrohm744).

Data were analyzed using the Kruskal-Wallis test and one-way ANOVA and differences between means were tested by Duncan's test (Zar, 1996).

Results

In the current study, safflower seed, wheat straw and corn silage samples contained 12.16, 3.19 and 8.13% crude protein, 32.76, 1.68 and 3.11% ether extract, 19.83, 43.7 and 24.61% crude fiber, 32.52, 59.11 and 31.21% acid detergent fiber (ADF) and 44.20, 81.18 and 52.08% neutral detergent fiber (NDF), respectively.

Total safflower seed in the diet was adjusted to be 0 (control), 1 (S-I), 2 (S-II), 3 (S-III) kg/d of intake. A significant reduction in milk yield (Table 2) was observed (C = 13.39±0.23, S-I = 12.94±0.26, S-II = 13.46±0.24 and S-III = 11.83±0.52 kg/d) in the S-III group ($P<0.01$). The highest milk yield was obtained by the S-II group, but statistically similar to the C group. Diets had no significant effect on the percentage of milk fat (C = 3.99±0.18, S-I = 4.09±0.16, S-II = 3.87±0.35 and S-III = 3.75±0.30).

The effects of SS on rumen TVFA, ammonia and ruminal pH, blood serum beta carotene, vitamin A, serum urea and plasma vitamin E in dairy cows were not statistically different among groups (Table 3).

Table 2 - Average milk yields and milk fat

	Groups				P-value
	C	S-I	S-II	S-III	
Milk yield (kg/d)	13.39b±0.23	12.94ab±0.26	13.46b±0.24	11.83a±0.52	**
Milk fat (%)	3.99±0.18	4.09±0.16	3.87±0.35	3.75±0.30	NS

** a,b, means followed by different letters in the same row differ (P<0.01).
NS - not significant.
n:16.

Table 3 - Effect of safflower seed on rumen TVFA, ammonia and ruminal pH, serum beta-carotene, vitamin A, serum urea and plasma vitamin E in dairy cows

	Groups				P-value
	C	S-I	S-II	S-III	
Serum β-carotene (µmol/L)	0.95±0.18	1.00±0.25	1.16±0.42	1.19±0.41	NS
Serum vit. A (µmol/L)	0.87±0.12	1.01±0.12	1.24±0.20	1.26±0.34	NS
Plasma vit. E (µmol/L)	117.76±19.09	128.34±9.89	142.14±14.03	146.65±14.03	NS
Serum urea (mmol/L)	12.83±1.13	13.96±0.94	15.32±0.80	14.54±1.34	NS
Rumen TVFA (mmol/L)	133.75±8.94	108.00±8.84	114.50±8.44	119.88±8.13	NS
Rumen ammonia (mmol/L)	8.85±1.14	10.52±3.75	12.19±1.51	15.79±1.44	NS
Rumen pH	5.85±0.24	6.25±0.24	6.21±0.30	6.14±0.23	NS

NS - not significant.
n:16.

Discussion

With the increased quantity of safflower seed in the diet, the fat content in the diet also increased. The fat (ether extract) contents of concentrates were 3.07, 6.44, 10.86, 13.47% for treatments control, S-I, S-II and S-III, respectively.

Markus et al. (1996) replaced a part of the dry matter of a diet fed to dairy cows by whole sunflower seed and tallow in their experiment, in which three diets were evaluated in relation to a barley-based diet. To provide the same amount of oil in the basal diet, 2.7% tallow and 7.1% whole sunflower seed (experimental diets) were added. The milk yields of the groups were 34.4, 34.6, and 35.5 kg/d respectively, and were not influenced by diet. They also reported that inclusion of whole sunflower seeds as a source of fat leads to a similar result to productions of cows fed traditional diets. Petit (2003) investigated the effects of feeding formaldehyde-treated flaxseed or sunflower seed on digestion, milk production, milk composition, and blood composition of dairy cows. They reported that untreated whole flaxseed was readily accepted by dairy cows and had no negative effect on milk production. Moreover, feeding up to 30% of sunflower seed in the dry matter (DM) had no effect on milk production. These findings were contradictory to the findings of our research. In our experiment, intake of 3 kg safflower per day decreased milk production. Milk production decreased in the S-III group compared with the control group. All other groups had statistically similar milk yield. The S-II group produced more milk than other groups but it was not statistically significant.

Bottger et al. (2002) determined the effects of supplemental safflower seeds high in linoleic or oleic acid on cow BW change, body condition score, milk production, milk composition and some serum metabolites. Safflower seed supplements were formulated to provide 5% of DMI as fat. Milk fat was not different at d 30; however, at d 60 and d 90, milk fat was greater (P<0.05) in control and oleate-supplemented cows than linoleat-supplemented cows. Oleate supplementation increased milk fat at d 60. But contrarily to this finding, in the present study, milk fat level was not affected by dietary safflower supplementation. This may be because the duration of the lactation period (d 270+) is different and the safflower seed produced in Turkey are usually linoleic acid-rich varieties**.** Godfrey and Dhiman (2006) and Dschaak (2009) reported that the optimum level to feed safflower seed must be identified when it is fed as a whole seed. Similar to these findings, too much safflower seed supplementation in lactating dairy diets had negative impacts on lactation performance in our study.

Generally, the recommended amount of fat intake increases the level of milk fat. However, Magdus et al. (1988) stressed that the physical form of oil is more important than the amount. Saturated, stabilized or protected fats are more efficient. Moreover, the feeding of unsaturated fats or large quantities of fat decreased the milk fat level. Nevertheless, Grummer (1991) reported that feeding free oil depressed

Table 4 - Phenotypic correlation coefficients among some blood and rumen parameters (rp)

Parameter		Vitamin A	Vitamin E	Serum urea	TVFA	Rumen ammonia	Rumen pH
Beta carotene	Pearson correlation	0.609*	0.574*	–0.275	–0.593*	–0.024	0.653**
	P-value	0.012	0.020	0.303	0.016	0.931	0.006
Vitamin A	Pearson correlation		0.442	–0.173	–0.412	0.446	0.528*
	P-value		0.087	0.522	0.113	0.083	0.036
Vitamin E	Pearson correlation			0.128	–0.195	0.180	0.048
	P-value			0.637	0.470	0.504	0.859
Serum urea	Pearson correlation				0.042	0.311	–0.199
	P-value				0.877	0.241	0.461
TVFA	Pearson correlation					–067	–0.900**
	P-value					0.807	0.000
Rumen ammonia	Pearson correlation						0.020
	P-value						0.941

n: 16, * P<0.05, ** P<0.01.

milk fat yield, but feeding oil as part of oilseeds did not change the milk-fat yield. In the present study, diets had no significant effect on milk fat, therefore milk fat tended to decrease in cows with increasing SS supplementation. This may be associated with the higher level of fat in the experimental diets compared with control diet.

Blood and rumen parameters of all treatment groups were statistically similar. However, there was an upward trend in beta-carotene, vitamin A and E levels when the amount of the SS increased in the diet. This may be related to the oil level in the diets. Fat in the diets helps the absorption of fat-soluble vitamins. The reason for this may be because the cows in our study were fed high-quality corn silage possibly with beta-carotene and tocopherol. Interaction between SS oil and vitamin levels may explain the non-linear increase.

The concentrations of rumen ammonia tended to increase, but this difference was not statistically significant. Hino et al. (1993) reported that addition of safflower oil to a growth medium of *in vitro* rumen bacteria culture depressed the bacterial growth above 200 mg/L. They also reported that addition of beta-carotene and tocopherol to the medium increased bacterial growth. Ammonia in the rumen is used by bacteria for microbial protein synthesis. If the bacterial population decreases, the usage of ammonia is reduced. In the current study, linoleic acid in the SS may have reduced the bacterial population in the rumen.

The results indicated that serum beta carotene was highly correlated with vitamin A, vitamin E, TVFA (P<0.05) and rumen pH (P<0.01). Serum vitamin A highly correlated with rumen pH (P<0.05). This finding can be related to the oil contents of safflower seed. Fat-soluble vitamins from concentrate diets and corn silage may more effectively absorb in SS groups than those in control group (Table 4).

The study of Dschaak et al. (2010) clearly demonstrated that it was highly possible to use Nutrasaff safflower seed as a means of fat supplementation to lactating dairy cows without negative impact on lactation performance, if added at less than 3% of dietary dry matter. However, our study yielded results showing that there is a negative effect in group SIII. Far more dietary safflower seed was used (approximately 20% of the dietary dry matter of group SIII is safflower seed) in this study. This negative effect stemmed from the excessive amount of fat supplied.

Conclusions

Short-time feeding of of up to 2 kg/d safflower seed has no negative effects on the milk yield, milk fat, and some serum parameters, but 3 kg reduces milk production. Safflower seed can be fed safely at the end stage of lactation in dairy cows at up to two kilogram daily. Further studies may be needed to determine whether high concentrations of safflower seed can be fed safely for longer periods.

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