Effects of sunflower oilseed supplementation on fatty acid profile and milk composition from Holstein cows

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ABSTRACT. Two groups of animals (control diet and control diet supplemented with sunflower) were evaluated in three lactation phases (0 to 60; 61 to 90; and 91 to 120 days in milk). During phase I, cows fed without sunflower showed higher concentrations of capric, lauric, palmitic, and araquidic acids. Only stearic acid had low concentrations during this phase. Cows fed with sunflower presented a reduction in the concentration of saturated fatty acids during phases I and II. During phase III, there was a concentration decrease of lauric, miristic, and pentadecilic acids in the sunflower-fed cow's milk Adding sunflower to cow's di*et al*tered the profile of milk fatty acids. Milk nutrients were not altered by adding sunflower to the diet, excepting milk protein concentration, which registered decreases of 3.6% in phase I, 3.4% in phase II and 6.7% in phase III.

Key words: concentrate, diet, milk quality, milk protein.

RESUMO. Efeitos da suplementação com grãos de girassol sobre o perfil dos ácidos graxos e composição do leite de vacas da raça holandesa. Dois grupos de animais (dieta base e dieta base acrescida girassol) foram avaliados em três fases da lactação (0 a 60; 61 a 90 e 91 a 120 dias de lactação). Na fase I, o tratamento sem girassol obteve maior concentração dos ácidos graxos cáprico, láurico, palmítico e araquídico, sendo a concentração do esteárico reduzida. Nas fases I e II houve redução da concentração dos ácidos graxos saturados no tratamento com girassol. Na fase III houve redução dos ácidos graxos láurico, mirístico e pentadecílico no tratamento com girassol. Verificou-se que a adição de girassol à dieta das vacas alterou o perfil dos ácidos graxos da gordura do leite. Os nutrientes do leite não foram alterados pela adição dos grãos de girassol na dieta, exceto no caso da proteína que houve uma diminuição na concentração de 3,6% na fase I, 3,4% na fase II e 6,7% na fase III.

Palavras-chave: concentrado, dieta, qualidade do leite, proteína do leite.

Introduction

The desire to improve bovine milk quality with increased concentrations of mono and polyunsaturated fatty acids, with positive economic and health perspectives (Delbecchi *et al*., 2001) has motivated this study. Human health has benefited of milk's increased nutritional value due to modifications of milk's fatty contents, associated with increased fatty acids distribution, as may be exemplified by the decrease of coronary cases (DePeters *et al*., 2001).

Milk quality and fatty composition are influenced by innumerous factors such as: fiber quantity and quality, concentrate:forage ratio, site and level of starch degradation, diet composition of fatty acids, and protection degree of ruminal degradation obtained from the supplement of fatty acids saturation (Tackett *et al*., 1996). Banks *et al*. (1980), have demonstrated that milk fatty quality can be influenced by lipids supplementation in cows feed. Vegetable and animal lipids supplement, ministered for lactating cows, resulted in oleic acid concentration increase and palmitic acid concentration decrease.

Long-chained unsaturated fatty acids, such as oleic, linoleic and linolenic acids, are hydrogenized and isomerized in the rumen (Delbecchi *et al*., 2001). Hydrogenation is completed by the activity of desaturase, mostly located in the mammary gland, but also in the intestines (Grummer, 1991). Methods to reduce fatty acids hydrogenation in the rumen include the utilization of protective measures that make these acids unavailable to bacteria in the rumen. Such methods include extrusion, pellets, treatment with

formaldehyde, calcium salts, and a mixture of animal and vegetable fat, in addition to the usage of oily whole oilseeds (Ashes *et al*., 1997).

Delbecchi *et al*. (2001) have verified that the addition of oily oilseeds to lactating cattle feed resulted in short-chained fatty acids (C10 – C16) concentration decrease, with a corresponding increase in the concentration of long-chained fatty acids, such as stearic (C18:0) and oleic (C18:1) acids. The addition of fat sources to lactating cows diet could restrict protein and microbiological synthesis, resulting in a reduced protein flow to the small intestine (Palmquist *et al*., 1993). Consequently, there is a reduction of the flow of amino acids to the mammary gland and a low protein milk content (DePeters and Cant, 1992; Dhiman *et al*., 2001).

The aim of this study was to evaluate the composition of milk produced by Holstein cows under a diet containing sunflower oilseeds.

Material and methods

This experiment was realized in *Fazenda Santa Rita* (Agropecuária Agrindus SA), *Descalvado*, state of *São Paulo*, Brazil. All animals were confined in pens under free stall system and received a control diet (total mixed feed). A diet (Table 1) was given to two groups; in group 1, animals were kept on a control diet and animals from group 2 received the same diet, but 1kg of sunflower oilseeds (Catisol variety) was also administered to each animal.

Table 1. Comparative nutritional composition of sunflower (*Helianthus annus*) oilseeds and experimental diets to which or no sunflower supplemented.

Nutrients	Sunflower oilseeds (%)	Diet (%)[1]	
		Without Sunflower	Sunflower Supplemented
Dry matter	94.1	52.3	53.2
Crude protein	25.6	17.1	17.3
Neutral detergent fiber	53.7	34.0	34.5
Ether extract	42.4	3.8	4.3
Calcium	0.2	1.1	1.1
Phosphorous	0.5	0.4	0.4

[1]Ingredients: corn, soybean oilseeds, soybean meal, mineral and vitamin supplement, molasses, salt, urea, citric pulp, cotton seed, corn silage, coast cross grass hay, alfalfa hay, and pre-dried Tifton grass.

The feed was divided into four meals. The second daily fraction was mixed to the first; the third fraction was mixed to the remains of the previously composed fraction; and the last was mixed to the remains of the composed third fraction. The last composed fraction was available all night long. The following day, feed not eaten was removed, the trough cleaned and replaced with fresh ration. To achieve maximum oilseed intake, sunflower oilseeds were only administered to the first meal-fraction each day. Small samples of each meal were collected, stored and mixed with the other samples of the same day. This compound sample was utilized to determine qualitative food analysis.

The study was realized with lactating cows between the second and the fifth lactation, and randomly allocated to groups. The experimental period started with the birth of calves. The initial lactating period was divided into three phases: Phase I (0 to 60 days in milk); Phase II (61 to 90 days in milk); and Phase III (91 to 120 days in milk).

Cows were milked three times a day, using an automatic milk recording system (Alfa Laval SA). Daily individual milk samples were obtained from each cow, from each treatment and from each lactating phase. The samples were stored and analyzed to determine the fatty acid profile and the chemical parameters. Milk samples were collected at days 45, 75, and 100 days of lactation, which means one sample for each phase. Total solids, crude protein, lactose, and the somatic cell count of all milk samples were determined at the Laboratory of Physiology, *Departamento de Produção Animal, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba*, State of *São Paulo*, Brazil.

The extraction of total fatty acids from milk samples was performed through clotting and centrifugation, described by ISO (1978), modified by Murphy *et al*. (1995). Identification of fatty acids was done at the Chromatography Laboratory, *Departamento de Química, Universidade Estadual de Maringá*. The transmethylation of fatty acids in solution of n-heptane and KOH/methanol was done through the method described by International Standards Organization (1978). The fatty acids methyl esters (FAME) were analyzed in a Shimadzu 14A (Japan) gas chromatograph, equipped with a flame ionization detector (FID) and fitted with silica capillary column (50m length, 0.25 cm i. D., and 0.2μm of Carbowax 20M). Column temperature was programmed to increase temperature from 150ºC to 240ºC at a rate of 2ºC/min. Injector and detector temperature were 220ºC and 245ºC, respectively. Carrier gas was hydrogen (1.2mL/min) and the make-up gas was nitrogen (30mL/min). The split used was 1/100. Peak areas were determined using the CG-300 computing integrator and FAME identification was made by comparison with the retention times of the known standards from Sigma (USA).

The following mathematical equation was used to analyze the effect of sunflower addition in the composition of each milk sample at 45, 75, and 100 days of lactation:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where Y_{ij} is the observation referent to repetition j and treatment i; μ, general average; G_i, effect of treatment i (with or without sunflower); and e_{ij}, random error associated with each sample.

In each lactation phase was utilized a different number of animals per treatment (Table 2). This procedure was necessary because some animals were excluded for sanitary reason, during the experimental phase.

Table 2. Number of animals allocated in each treatment for each lactation phase.

Lactation phase	Diet		Total
	Without Sunflower	Sunflower Supplemented	
I	19	18	27
II	19	16	35
III	18	16	34

Data were analyzed with the GLM procedure of SAS Statistical Package (SAS, 1996); means were compared by the Turkey test, using a probability of 5%.

Results

The profiles of fatty acids obtained from the milk samples in Phase I, II, and III are shown in Tables 3, 4, and 5, respectively.

During Phase I (Table 2), there were alterations in the concentration of the following saturated fatty acids: caprilic (C8:0), capric (C10:0), lauric (C12:0), miristic (C14:0), palmitic (C16:0), stearic (C18:0), araquidic (C20:0) and in the sum of saturated fatty acid (SATFA). A high concentration ($P< 0.05$) of these fatty acids was observed in milk from those animals fed without sunflower, excepting stearic acid, which showed and inverted pattern. The concentrations of fatty acids C8:0, C10:0, C12:0, C14:0, C16:0, C20:0 and SATFA observed in the fatty portion of milk obtained from the animals fed without sunflower oilseeds were increased by 16.8%, 24.4%, 21.2%, 13.6%, 9.2%, 42.3% and 5.8%, respectively.

The concentration of the stearic and oleic/elaidic acid observed in the milk from these animals was 24.3% and 15.3% lower, respectively, when compared to the average concentration of milk from animals that received sunflower grain in their diet (Table 3).

Table 3. Dietary averages, minimum significant differences (MSD), and standard errors of mean (SEM) observed from the profile of milk fatty acids produced by Holstein cows during Lactating Phase I (0-60 days) relative to the respective treatment (control or sunflower supplemented) administered in the diet.

Fatty acids		Diet (%)		MSD	SEM
Usual Nomenclature	Symbol	Control	Sunflower Supplemented		
Butyric	C4:0	2.23	2.53	0.41	0.38
Caprioic	C6:0	2.08	1.94	0.27	0.16
Caprilic	C8:0	1.46*	1.25	0.18	0.07
Capric	C10:0	3.26*	2.62	0.48	0.52
Lauric	C12:0	3.31*	2.73	0.45	0.43
n-Tridecilic	C13:0	0.14	0.12	0.88	1.73
Miristic	C14:0	10.02*	8.82	0.21	0.09
Miristoleic	C14:1(9) 9	0.78	0.66	0.06	0.01
Pentadecilic	C15:0	1.04	0.98	0.23	0.11
cis-10-Pentadecanoic	C15:1	0.16	0.14	0.02	0.00
Palmitic	C16:0	25.90*	23.72	1.55	5.37
Palmitoleic	C16:1(9) 7	1.77	1.64	0.44	0.41
Margaric	C17:0	0.46	0.46	0.05	0.01
cis-10-Heptadecanoic	C17:1	0.32	0.27	0.09	0.02
Stearic	C18:0	10.60	13.18*	1.52	5.21
Oleic/Elaidic	C18:1/C18:1, trans-9	23.43	27.01*	2.74	16.87
Linoleic/Linolelaidic	C18:2/C18:2, trans-9, 12	3.29	3.53	0.29	0.19
¯Linolenic	C18:3(9, 12, 15)3	0.45	0.42	0.07	0.01
-Linolenic	C18:3(6, 9, 12) 6	0.57	0.61	0.15	0.05
Araquidic	C20:0	0.37*	0.26	0.11	0.03
Saturated	SATFA	61.51*	58.15	3.15	22.31
Monounsaturated	MUFA	26.06	29.40*	2.93	19.26
Polyunsaturated	PUFA	4.31	4.56	0.38	0.33
Total		91.64	92.89	2.53	7.14

Table 4. Dietary averages, minimum significant differences (MSD), and standard errors of mean (SEM) observed from the profile of milk fatty acids produced by Holstein cows during Lactating Phase II (61-90 days) relative to respective treatment (control or sunflower supplemented) administered in the diet.

Fatty acids		diet (%)		MSD	SEM
Usual Nomenclature	Symbol	Control	Sunflower Supplemented		
Butyric	C4:0	2.45	2.76	0.51	0.55
Caprioic	C6:0	2.20	2.21	0.33	0.23
Caprilic	C8:0	1.52	1.37	0.25	0.13
Capric	C10:0	3.26	2.81	0.55	0.64
Lauric	C12:0	3.33*	2.72	0.49	0.52
n-Tridecilic	C13:0	0.14*	0.11	0.03	0.00
Miristic	C14:0	10.18*	8.79	0.97	1.96
Miristoleic	C14:1(9) 9	0.74	0.62	0.13	0.04
Pentadecilic	C15:0	1.06*	0.82	0.15	0.05

cis-10-Pentadecanoic	C15:1	0.17	0.17	0.06	0.01
Palmitic	C16:0	26.81*	23.36	2.03	8.65
Palmitoleic	C16:1(9) 7	1.62*	1.39	0.23	0.11
Margaric	C17:0	0.46*	0.38	0.04	0.00
cis-10-Heptadecanoic	C17:1	0.19	0.17	0.03	0.00
Stearic	C18:0	10.63	12.22*	1.58	5.27
Oleic/Elaidic	C18:1/C18:1, trans-9	24.02	26.59*	2.53	13.45
Linoléico/Linolelaidico	C18:2/C18:2, trans-9, 12	3.94	3.99	0.37	0.29
¯Linolenic	C18:3(9, 12, 15)3	0.47	0.38	0.11	0.02
-Linolenic	C18:3(6, 9, 12) 6	0.65	0.74	0.13	0.04
Araquidic	C20:0	0.23	0.29	0.06	0.01
Saturated	SATFA	62.26*	57.83	3.44	24.89
Monounsaturated	MUFA	26.75	28.94	2.58	13.98
Polyunsaturated	PUFA	5.07	5.11	0.44	0.41
Total		94.07	91.89	2.69	15.20

Table 5. Dietary averages, minimum significant differences (MSD), and standard errors of mean (SEM) observed from the profile of milk fatty acids produced by Holstein cows during Lactating Phase III (91-120 days) relative to the respective treatment (control or sunflower supplemented) administered in the diet.

Fatty acids		Diet (%)		MSD	SEM
Usual Nomenclature	Symbol	Control	Sunflower Supplemented		
Butyric	C4:0	2.36	2.69	0.85	1.48
Caprioic	C6:0	2.17	2.17	0.50	0.52
Caprilic	C8:0	1.49	1.41	0.26	0.13
Capric	C10:0	3.41	3.01	0.44	0.39
Lauric	C12:0	3.50*	2.98	0.37	0.27
n-Tridecilic	C13:0	0.14	0.12	0.03	0.00
Miristic	C14:0	10.62*	9.59	0.63	0.82
Miristoleic	C14:1(9) 9	0.85	0.74	0.17	0.06
Pentadecilic	C15:0	0.99*	0.78	0.13	0.03
cis-10-Pentadecanoic	C15:1	0.18	0.20	0.04	0.00
Palmitic	C16:0	27.20	25.58	2.17	9.58
Palmitoleic	C16:1(9) 7	1.63	1.51	0.29	0.17
Margaric	C17:0	0.44	0.42	0.06	0.01
cis-10-Heptadecanoic	C17:1	0.19	0.20	0.05	0.00
Stearic	C18:0	9.93	12.08*	1.49	4.54
Oleic/Elaidic	C18:1/C18:1, trans-9	22.84	25.15	2.34	11.21
Linoléico/Linolelaidic	C18:2/C18:2, trans-9, 12	4.13	3.91	0.63	0.81
¯Linolenic	C18:3(9, 12, 15) 3	0.31	0.34	0.09	0.01
-Linolenic	C18:3(6, 9, 12) 6	0.61	0.64	0.12	0.03
Araquidic	C20:0	0.26	0.21	0.06	0.01
Saturated	SATFA	62.51	61.04	2.59	13.68
Monounsaturated	MUFA	25.69	27.80	2.42	11.96
Polyunsaturated	PUFA	5.05	4.89	0.69	0.98
Total		93.25	93.73	1.72	6.02

A significant difference ($p < 0.05$) for the sum of monounsaturated fatty acids (MUFA) was observed (Table 3). MUFA concentration observed in milk derived from cows fed with sunflower grain was 12.8% superior. This higher MUFA concentration is directly related to a higher (15.3%) concentration of oleic/elaidic fatty acid in cows that received sunflower oilseeds.

During Phase II (Table 4), there was a reduction in the concentration of saturated fatty acids from milk produced by cows supplemented with sunflower oilseeds. This study has registered a decrease on concentrations of the following fatty acids: lauric (22.4%), n-tridecilic (27.3%), miristic (15.8%), pentadecilic (29.3%), palmitic (14.8%), and margaric (21.1%), for cows fed with sunflower. The only exception were stearic and oleic/elaidic acids, which demonstrated comparatively high values: 15.0% and 10,7%, respectively, for cows fed with sunflower. The total of saturated fatty acids observed in milk from sunflower-fed cows was 7.7% lower than in animals that did not receive this supplement. The standard error of this average was 13.45, indicating immense statistical variation.

In Phase III (Table 5), a significant reduction in the concentration of saturated fatty acids lauric (17.5%), miristic (10.7%), and pentadecilic (26.9%) was observed in the milk of sunflower-fed animals. However, stearic acid demonstrated an inverse pattern, with an increase of 21.7%.

Table 6 shows that most of the nutrients contained in milk were not altered by the addition of sunflower to the diet of these Holsteins cows, except for a reduction in the protein concentration ($p < 0.05$).

Table 6. Average (in percentage) concentrations of fat, protein, lactose, total solids, and somatic cell count of milk produced by Holstein cows supplemented or not with sunflower during the lactating phases evaluated.

Nutrients	Treatment		MSD	SEM
	Control	Sunflower supplemented		
	Phase I			
Fat (%)	3.1	3.2	0.40	0.39
Protein (%)	2.8*	2.7	0.15	0.05
Lactose (%)	4.6	4.7	0.17	0.07
Total solids (%)	11.4	11.4	0.57	0.78
SCC	20.9	12.9	14.23	493.82

	Phase II			
Fat (%)	3.1	2.9	0.49	0.57
Protein (%)	2.9*	2.8	0.15	0.05
Lactose (%)	4.6	4.6	0.16	0.06
Total solids (%)	11.5	11.1	0.64	0.96
SCC	14.0	11.2	7.76	142.89
	Phase III			
Fat (%)	3.0	3.3	0.57	0.65
Protein (%)	3.0*	2.8	0.15	0.05
Lactose (%)	4.6	4.6	0.18	0.06
Total solids (%)	11.4	11.6	0.69	0.93
SCC	16.2	10.3	8.02	127.47

SCC = somatic cells count, square root of the expected value; MSD = minimum significant difference; SEM = standard error mean.

Discussion

Experimental data from this study demonstrated that the concentration of the saturated miristic (C14:0) fatty acid was lower ($p<0.05$) in all lactation phases when sunflower oilseeds were administered (Table 2, 3, and 4). Cows not fed with sunflower during the lactating phases examined demonstrated similar concentrations of this saturated fatty acid, as previously reported by Ashes *et al*. (1997), who observed concentrations between 10 and 12%.

The concentration of palmitic (C16:0) and palmitoleic (C16:1) acids observed in this study are similar to those described by Delbecchi *et al*. (2001), who used canola oilseeds protected from ruminal fermentation by formaldehyde and obtained similar results with stearic and oleic acids. This may indicate that the skin of sunflower offers better protection from ruminal degradation than the treatment with formaldehyde. An increase in the concentration of saturated fatty acids, principally miristic (C14:0) and palmitic (C16:0), is known to elevate the concentration of low density plasmatic lipoproteins and, consequently, the risk of coronary disease (Noakes *et al*., 1996). Modern lifestyle is extremely sedentary, predisposing humans to an increase in the serum concentration of low density lipoproteins resulting in higher arteriosclerosis risk. Therefore, any reduction of unwanted saturated fatty acids, as observed in this study, is fundamental to reduce the risk of coronary diseases.

The elevated concentrations of stearic (C18:0) and oleic (C18:1) acids observed in this study are similar to the results described by Foncat *et al*. (1998) and Tymchuk *et al*. (1998), who used canola oilseeds protected from ruminal degradation. The elevated concentration of the monounsaturated acid oleic (C18:1) observed in this study is directly related to a corresponding increase of its precursor, stearic acid (C18:0). This reaction is catalyzed by the enzyme delta-9-esteroil-CoA dessaturase, located in the mammary gland (Delbecchi *et al*., 2001).

Even when an increase in the concentration of stearic fatty acid was observed in milk obtained from cows fed with sunflower oilseeds (Table 2), the total concentrations of saturated fatty acids in this group was relatively lower ($p < 0.05\%$). The finding of relatively low concentrations of fatty acids in milk obtained from cows fed with sunflower is of great importance to human nutrition, since fatty acids are related to several health problems (Andrade *et al*., 1994).

Evidence of the inversely proportional effect between the concentration of polyunsaturated fatty acids found in the diet, blood and/or tissue and a reduced incidence of experimental heart diseases in tissue culture and clinical trials in humans and animals has already been established (Uauy and Valenzuela, 2000). The preventive action of polyunsaturated fatty acids in heart diseases is due to several actions: prevention of arrhythmias, production of prostaglandins and leukotrienes with anti-inflammatory action, and inhibition of cytokine synthesis that increases inflammation and platelets formation. These components stimulate endothelial-derived nitric oxide, which relaxes and soothes the musculature, promoting endothelial repair and plasmatic lipids reduction, especially triglycerides e VLDLs.

The reduction in protein milk concentration has been described by Park (1998). This author observed a significant alteration of milk protein concentration when sunflower oilseeds given at 14% were included in the diet. Other authors (Drackley and Schingoethe, 1986) have indicated that a reduction in the protein concentration of milk in animals should not occur, since the amino acids balance in sunflower protein is better than in soybean, proposing a higher biological value of this oilseed protein, thereby being available, in larger quantities, in the mammary gland.

However, the reduced concentration of milk protein observed in this study may be directly related to the inhibition of ruminal microbial protein synthesis, resulting in a reduced flow of amino acids to the small intestine and mammary gland and a consequent fall in milk protein concentration (DePeters and Cant, 1992; Palmquist *et al*., 1993; Dhiman *et al*., 2001).

An insignificant reduction in the number of somatic cells from the milk obtained from sunflower-fed cows during all lactating phases was observed in this study. Reduction of somatic cells has been associated to an increase in the concentration of polyunsaturated fatty acids in the diet, blood and/or tissues with a subsequent increase in tissue repair and health of the mammary gland (Uauy and Valenzuela, 2000).

Conclusion

This study has demonstrated that the supplementation of sunflower oilseeds to the diet of Holstein cows resulted in an alteration of the milk fatty acids profile during the three lactating phases

evaluated, increasing the concentration of unsaturated fatty acids and decreasing the saturated ones.

Sunflower oilseeds added to the diet decreased milk protein concentration and caused a non-statistical reduction, but repeated in all lactating phases, in the number of somatic cells from the milk.

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