



Effect of storage on fatty acid profile of butter from cows fed whole or ground flaxseed with or without monensin¹

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ABSTRACT - Eight Holstein cows with body weight 570 ± 43 kg and 60 ± 20 lactation days were distributed in a double Latin square design with four 21-day periods to determine the effects of feeding ground or whole flaxseed with or without monensin supplementation (0.02% on a dry matter basis) on fatty acid profile of butter stored for 15 and 45 days. Ground flaxseed supply, in comparison to whole flaxseed, reduced relative percentages of 16:0, cis7-16:1, 17:0, and cis10-17:1 but it increased those of cis9,trans11-18:2, cis3-18:3, and omega 3 fatty acids in butter fat, reducing relative percentage of medium-chain fatty acids and increasing the content of polyunsaturated fatty acids. Supplementation with monensin increased relative percentages of cis9,trans11-18:2 and tended to increase relative percentage of 17:0 and decrease that of saturated fatty acids in butter. Butter from cows fed diet with monensin presented lower relative percentages of cis 6-20:4. Relative percentages of cis 9-16:1, cis10-17:1, 18:0, trans11-18:1, cis9-18:1, cis3-18:3, cis6-20:4 in butter stored for 15 days were higher than those stored for 45 days and the relative percentages of cis3-20:5 tended to decrease with the increase of storage period. As a result, relative percentages of saturated fatty acids and medium-chain fatty acids increased with storage time, while those of monounsaturated and long-chain fatty acids decreased. Butter enriched with polyunsaturated fatty acids may have a shorter shelf life due to the negative effect of storage on fatty acid profile which may cause oxidation and rancidity.

Key Words: butter, dairy cow, fatty acid, flaxseed, oxidation

Efeito do armazenamento sobre o perfil de ácidos graxos da manteiga de vacas alimentadas com dietas contendo linhaça inteira ou moída, com ou sem monensina sódica

RESUMO - Oito vacas da raça Holandesa com 570 ± 43 kg de peso vivo e 60 ± 20 dias de lactação foram distribuídas em delineamento quadrado latino duplo, com quatro períodos de 21 dias, para avaliar o fornecimento de linhaça inteira ou moída, com ou sem suplementação de monensina sódica (0,02% na MS), sobre o perfil de ácidos graxos da manteiga armazenada por 15 e 45 dias. O fornecimento de linhaça moída, em comparação ao de linhaça inteira, diminuiu as porcentagens relativas de 16:0, cis7-16:1, 17:0, e cis10-17:1, mas aumentou cis9,trans11-18:2, cis3-18:3, e ômega-3 na manteiga, reduzindo a porcentagem relativa de ácidos graxos de cadeia média e aumentando o conteúdo de ácidos graxos poliinsaturados. A suplementação com monensina aumentou as porcentagens relativas de cis9,trans11-18:2 e tendeu a aumentar a porcentagem relativa de 17:0 e diminuir ácidos graxos saturados na manteiga. A manteiga das vacas que receberam monensina na dieta apresentou menor porcentagem relativa de cis6-20:4. As porcentagens relativas de cis9-16:1, cis10-17:1, 18:0, trans11-42 18:1, cis9-18:1, cis3-18:3, cis6-20:4 na manteiga armazenada por 15 dias foram maiores que naquela armazenada por 45 dias e as porcentagens relativas de cis3-20:5 tenderam a diminuir com o aumento do período de armazenamento. Em consequência, as porcentagens relativas de ácidos graxos saturados e de cadeia média aumentaram com o tempo de armazenamento, enquanto ácidos graxos monoinsaturados e de cadeia longa diminuíram. A manteiga enriquecida com ácidos graxos poliinsaturados pode ter vida de prateleira mais curta, devido ao efeito negativo do armazenamento no perfil de ácidos graxos, que pode provocar oxidação e rancidez.

Palavras-chave: ácidos graxos, linhaça, manteiga, oxidação, vaca leiteira

Introduction

Some long chain polyunsaturated fatty acids (PUFA) are recognized for their potential effects on human health. For example, conjugated linoleic acid (CLA, *cis*9,*trans*11-18:2) shows antiatherogenic, immunomodulating, growth promoting, and lean body mass-enhancing properties (Pariza, 1997) while omega-3 fatty acids (FA) prevent tumor development (Willett, 1997) and certain cardiovascular diseases in human beings (Bang et al., 1976). Dietary feeding strategies may contribute to increase conjugated linoleic acid, omega-3, and other beneficial fatty acids in milk fat of dairy cows (Petit et al., 2004; Baer et al., 2001). Also, modifications in the profile of fatty acids may contribute to alter physical properties of milk fat such as improved butter spreadability (Enjalbert et al., 1997) as a result of higher and lower concentrations of *cis*-C18:1 and C16:0, respectively. However, ruminal biohydrogenation of dietary polyunsaturated fatty acids may be high as it was shown by the low transfer of polyunsaturated fatty acids from whole flaxseed into milk (Petit et al., 2005). Nonetheless, it has been shown that infusion of flaxseed oil in the abomasum, which avoids ruminal biohydrogenation of polyunsaturated fatty acids by bypassing the rumen, increases milk concentration of linolenic acid to 13.9% of total fatty acids as compared to 2.0% when whole flaxseed is supplied in the diet of dairy cows (Petit et al., 2002). Therefore, means to decrease ruminal biohydrogenation would contribute to increase the transfer of polyunsaturated fatty acids from flaxseed in milk, which may modify the physical properties and fatty acid profile of butter.

Monensin is known to decrease *in vitro* ruminal biohydrogenation of PUFA (Van Nevel & Demeyer, 1995) and to increase total CLA concentration *in vitro* (Fellner et al., 1997) and in milk of dairy cows (da Silva et al., 2007). Moreover, feeding ground compared with whole flaxseed increased concentrations of conjugated linoleic acid and *cis*3-18:3 in milk fat (da Silva et al., 2007). Unsaturated fatty acids are more reactive than the saturated, so they are more susceptible to oxidation and have a shorter shelf life (Rossell, 1989). In addition, oxidations of milk with higher concentrations of monounsaturated FA (MUFA) and polyunsaturated fatty acids are greater than that of milk with lower concentrations after three days of storage while there is no difference in oxidation when both milks are fresh (Hedegaard et al., 2006).

Therefore, means to decrease ruminal biohydrogenation, which have been studied for many researches in the last decade (Santos et al., 2010), would contribute to increase the transfer of polyunsaturated fatty acids from flaxseed in

milk and to modify the physical properties and fatty acid profile of butter.

Material and Methods

A total of 8 multiparous Holstein cows with average body weight 570 ± 43 kg and 60 ± 20 days in milk were assigned to a replicated 4×4 Latin square design to determine the effects of flaxseed processing and monensin supplementation on dry matter (DM) and fat concentrations and fatty acid profile of butter. Each experimental period consisted of 21 days and milk collection for butter production was carried out on day 16. The proceedings to obtain the four total mixed rations and the composition, also the information about the experiment conduction are described by da Silva et al. (2007).

On day 16 of each experimental period, milk was obtained from the two milkings and pooled within cow and period relative to production to obtain one composite milk sample (10 L) per cow per period for butter fabrication. Milk was kept at 4°C for 24 h to permit cream separation, then it was filtered and stored in plastic containers. Cream was immediately pasteurized at 75°C for 30 min and kept at 4°C for 20 h. An electric mixer (Churned Ciranda, ARNO S.A., São Paulo, Brazil), which was operated at the speed setting 2 at 4°C, was used to make butter in duplicate churnings of 200-300 g of cream from each one of the 32 milk samples for a total of 64 butter samples. Churning times varied from 30 to 50 min. Concentrations of dry matter and fat content were determined immediately. Butter samples were stored in closed plastic containers at 4°C until further determination of fatty acids profile, which was determined after 15 and 45 days of storage.

Dry matter of the butter was analyzed according to the procedure 934.01 (AOAC, 1990). Analysis of total fat in butter was determined according to the method of Bligh & Dyer (1959). Fatty acids in butter were methylated as described in da Silva et al. (2007).

All results were analyzed using the MIXED procedure of SAS (2000) within a 2×2 factorial arrangement of diets. Data on butter dry matter and fat were analyzed using a replicated 4×4 Latin square design with the following general model:

$$Y_{ijkl} = \mu + S_i + C_j(i) + P_k + T_l + e_{ijkl}$$

in which: Y_{ijkl} = the dependent variable; μ = overall mean; S_i = random effect of square ($i = 1$ to 2); $C_j(i)$ = random effect of cow within square ($j = 1$ to 4); P_k = period fixed effect ($k = 1$ to 4); T_l = diet fixed effect ($l =$ WFCO, WFMO, GFCO, GFMO); and e_{ijkl} = random residual error.

Data on butter fatty acid profile were analyzed following the same model for dry matter and fat, but analyzed as

repeated measurements (15 and 45 days of storage) using PROC MIXED (SAS, 2000). Experimental diets were compared to provide factorial contrasts: whole vs. ground flaxseed, with monensin vs. without monensin, and the interaction between flaxseed processing and monensin supplementation. Significance was declared at $P < 0.05$ and a trend at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

The chemical composition of the total mixed ration presented by da Silva et al. (2007) was generally similar among diets. There was no interaction between diets vs.

day ($P > 0.10$) except for unidentified fatty acids, where there was a trend ($P = 0.09$).

In general, there were no interactions between flaxseed processing and monensin supplementation on butter concentrations of individual fatty acids except for *trans*11-18:1 concentration that was twice higher ($P < 0.001$) in butter fat of cows fed ground flaxseed with monensin compared to butter fat of cows fed the other diets (Table 1), which agrees with the profile of fatty acids of milk produced from cows fed the same four diets (da Silva et al., 2007).

Feeding ground flaxseed compared to whole flaxseed decreased relative percentages of 16:0, *cis*7-16:1, 17:0 and *cis*10-17:1, and it increased those of *cis*9, *trans*11-18:2,

Table 1 - Fatty acid concentrations in butter (percentage of total fatty acids)¹

	Experimental diets				SE	Probability		
	Whole flaxseed	Whole flaxseed + monensin	Ground flaxseed	Ground flaxseed + monensin		Flaxseed	Monensin	Flaxseed × monensin
6:0	0.74	0.75	0.76	0.72	0.19	0.98	0.93	0.88
8:0	0.66	0.61	0.64	0.67	0.10	0.83	0.93	0.72
10:0	1.96	1.77	1.91	1.97	0.17	0.68	0.70	0.48
11:0	0.04	0.04	0.04	0.04	0.005	0.94	0.93	0.94
12:0	2.17	2.33	2.52	2.48	0.14	0.89	0.25	0.36
13:0	0.09	0.09	0.09	0.09	0.008	0.45	0.83	0.64
14:0	10.00	9.34	9.79	9.61	0.34	0.94	0.23	0.48
<i>cis</i> 10-15:1	0.90	0.97	0.86	0.90	0.04	0.15	0.16	0.66
<i>iso</i> 16:0	0.21	0.19	0.19	0.20	0.01	0.54	0.71	0.37
16:0	26.63	26.67	25.37	24.80	0.69	0.04	0.70	0.67
<i>cis</i> 9-16:1	0.18	0.21	0.18	0.19	0.015	0.49	0.18	0.74
<i>cis</i> 7-16:1	1.52	1.51	1.30	1.39	0.07	0.02	0.57	0.51
17:0	0.47	0.51	0.45	0.45	0.01	0.001	0.08	0.13
<i>iso</i> 17:0	0.57	0.57	0.51	0.57	0.02	0.16	0.12	0.18
<i>cis</i> 10-17:1	0.23	0.23	0.18	0.17	0.02	0.006	0.83	0.73
18 :0	16.44	16.56	17.97	15.47	0.75	0.77	0.13	0.10
<i>trans</i> 11-18:1	1.31	1.53	1.59	3.00	0.07	<0.0001	<0.0001	<0.0001
<i>cis</i> 9-18:1	26.94	27.17	26.82	26.40	0.79	0.57	0.90	0.68
<i>cis</i> 6-18:2	1.60	1.73	1.65	1.88	0.14	0.47	0.20	0.72
<i>cis</i> 9, <i>trans</i> 11-18:2	0.44	0.52	0.53	0.91	0.10	0.03	0.03	0.15
<i>cis</i> 12, <i>trans</i> 10-18:2	0.07	0.07	0.06	0.07	0.01	0.44	0.91	0.29
<i>cis</i> 3-18:3	0.76	0.77	1.08	0.86	0.07	0.01	0.17	0.12
<i>cis</i> 6-20:4	0.10	0.09	0.09	0.08	0.01	0.10	0.04	0.56
<i>cis</i> 3-20:5	0.06	0.05	0.06	0.06	0.01	0.37	0.67	0.83
<i>cis</i> 6-22:4	0.03	0.02	0.02	0.03	0.00	0.88	0.70	0.41
<i>cis</i> 3-22:6	0.06	0.06	0.07	0.06	0.01	0.59	0.23	0.62
Total <i>trans</i>	1.82	2.12	2.18	3.98	0.15	<0.0001	<0.0001	<0.0001
Unidentified	5.36	5.62	5.29	6.95	0.41	0.14	0.03	0.10
MUFA	31.09	31.62	30.92	32.04	0.85	0.88	0.34	0.74
PUFA	3.11	3.32	3.35	3.94	0.24	0.04	0.23	0.71
SFA	60.44	59.43	60.24	57.06	1.09	0.25	0.07	0.33
PUFA/SFA	0.05	0.06	0.06	0.07	0.01	0.08	0.13	0.60
n-3 ²	0.88	0.89	1.20	0.97	0.07	0.01	0.15	0.13
n-6 ³	1.72	1.85	1.76	1.99	0.14	0.52	0.23	0.72
n-6:n-3	2.00	2.17	1.53	2.01	0.10	0.01	0.007	0.12
SCFA	5.49	5.14	5.28	5.33	0.22	0.97	0.50	0.37
MCFA	38.28	37.87	37.33	36.80	0.48	0.05	0.33	0.90
LCFA	50.82	51.17	51.80	51.28	0.58	0.36	0.88	0.47

¹ Least squares means with pooled standard error (SE).

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.

² *Cis*3-18:3 + *cis*3-20:5 + *cis*3-22:6.

³ *Cis*6-18:2 + *cis*6-20:4 + *cis*6-22:4.

cis3-18:3, and omega-3 fatty acid in butter fat (Table 2). As a result, there was a decrease in relative percentages of medium chain fatty acids and an increase in relative percentages of polyunsaturated fatty acids following flaxseed grinding.

Monensin supplementation increased relative percentages of *cis9,trans11-18:2* and tended to increase relative percentage of 17:0 and decrease that of saturated fatty acids in butter fat ($P=0.08$ and 0.07 , respectively). Cows fed monensin had lower relative percentages of *cis6-20:4* in butter fat than those fed the control diets. Monensin supplementation increased relative percentages of unidentified fatty acids probably as a result of a greater production of *trans* fatty acid in the rumen with monensin

supplementation (Fellner et al., 1997). The n-6 to n-3 ratio in butter fat was significantly higher for cows supplemented with monensin but all ratios were below the 4:1 ratio recommended for reducing potential risk of coronary heart diseases (Sim, 1998). In general, the effects of flaxseed processing and monensin supplementation on the fatty acids profile of butter fat were similar to those obtained on the profile of fatty acids of milk (da Silva et al., 2007).

Relative percentages of 6:0, 8:0, 10:0, 12:0, and 14:0 were significantly greater in butter fat stored for 45 days than in that stored for 15 days and there was a trend for relative percentages of 11:0 and *iso16:0* to increase with storage time (Table 2). The shortest fatty acid (in particular 4:0 and 6:0), in the case of butter (flavour and odour) are mainly

Table 2 - Fatty acid concentrations in butter (percentage of total fatty acids) analysed in 15 and 45 days of storage¹

	Day		Probability SE	Day	Day × Diet
	15	45			
6:0	0.51	0.97	0.13	0.01	0.94
8:0	0.51	0.78	0.06	0.002	0.96
10:0	1.67	2.13	0.10	0.0005	0.96
11:0	0.04	0.05	0.00	0.06	0.62
12:0	2.35	2.62	0.08	0.0002	0.96
13:0	0.09	0.09	0.00	0.18	0.61
14:0	9.47	9.90	0.18	0.00	0.94
<i>cis10-15:1</i>	0.90	0.91	0.02	0.13	0.92
<i>iso16:0</i>	0.19	0.20	0.01	0.05	0.92
16:0	25.85	25.88	0.36	0.87	0.13
<i>cis9-16:1</i>	0.20	0.18	0.01	0.03	0.32
<i>cis7-16:1</i>	1.44	1.42	0.04	0.06	0.95
17:0	0.47	0.46	0.00	0.09	0.52
<i>iso 17:0</i>	0.56	0.55	0.01	0.40	0.77
<i>cis10-17:1</i>	0.21	0.19	0.01	0.01	0.62
18:0	16.88	16.34	0.39	0.03	0.71
<i>trans11-18:1</i>	1.89	1.82	0.04	0.02	0.23
<i>cis9-18:1</i>	27.42	26.24	0.43	0.001	0.94
<i>cis6-18:2</i>	1.72	1.71	0.08	0.97	0.47
<i>cis9,trans11-18:2</i>	0.61	0.58	0.05	0.13	0.31
<i>cis12,trans10-18:2</i>	0.07	0.06	0.00	0.10	0.21
<i>cis3-18:3</i>	0.89	0.84	0.04	0.02	0.18
<i>cis6-20:4</i>	0.10	0.09	0.00	0.04	0.52
<i>cis3-20:5</i>	0.06	0.05	0.00	0.08	0.53
<i>cis6-22:4</i>	0.026	0.024	0.00	0.37	0.28
<i>cis3-22:6</i>	0.06	0.06	0.00	0.47	0.70
Total trans	2.58	2.47	0.07	0.02	0.16
Unidentified	5.78	5.83	0.23	0.82	0.09
MUFA	32.07	30.77	0.46	0.001	0.89
PUFA	3.54	3.42	0.13	0.27	0.65
SFA	58.61	59.98	0.59	0.003	0.29
PUFA/SFA	0.06	0.06	0.00	0.18	0.60
n-3 ²	1.02	0.95	0.04	0.02	0.25
n-6 ³	1.84	1.82	0.08	0.81	0.49
n-6:n-3	1.86	2.00	0.06	0.04	0.29
SCFA	5.18	5.44	0.16	0.24	0.73
MCFA	36.41	38.73	0.33	<0.0001	0.15
LCFA	52.55	49.99	0.41	0.0001	0.69

¹ Least squares means with pooled standard error (SE).

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.

² *Cis3-18:3 + cis3-20:5 + cis3-22:6*.

³ *Cis6-18:2 + cis6-20:4 + cis6-22:4*.

responsible for rancid character (Walstra et al., 1999). In the present experiment, relative percentage of 6:0 increased with storage time, which may increase rancidity and decrease acceptability of butter by the consumer.

Butter fat stored for 15 days showed higher relative percentages of *cis*9-16:1, *cis*10-17:1, 18:0, *trans*11-18:1, *cis*9-18:1, *cis*3-18:3, *cis*6-20:4 compared to butter fat stored for 45 days and there was a gradually decrease of relative percentages of *cis*3-20:5 as they were stored for a longer period. As a result, relative percentages of saturated and medium-chain fatty acids increased overtime while those of monounsaturated fatty acids and long-chain fatty acids decreased, thus suggesting a conversion of saturated and medium-chain fatty acids from monounsaturated fatty acids and long-chain fatty acids due to oxidation of unsaturated fatty acids. (Hedegaard et al., 2006).

Butter was stored at cold temperature in the present experiment, which may have resulted in less significant changes in the profile of fatty acids. Heating of butter (e.g. for cooking) or storage for a longer period could have resulted in greater changes of profile of fatty acids as chemical changes in lipids are generally accelerated with the severity of the heat treatment and duration of storage (Herzallah et al., 2005). Relative percentage of conjugated linoleic acid in butter was not affected by storage as previously reported for fermented dairy products (Jenkins et al., 2003) and cheese (Herzallah et al., 2005).

Moreover, no changes in relative percentages of conjugated linoleic acid and other fatty acids in cheese enriched or not with polyunsaturated fatty acids have been reported over a 24-week aging period at 4°C (Allred et al., 2006) or 8°C (Coakley et al., 2007). On the other hand, Rodriguez-Alcala & Fontecha (2007) reported an increase in relative percentages of 16:0, 18:0 and saturated fatty acid and a decrease in those of polyunsaturated fatty acids in conjugated linoleic acid-fortified cheese after 10 weeks of refrigerated storage. Therefore, the effects of storage on profile of fatty acids dairy products remain unclear.

Flaxseed processing had no effect on butter fat concentration (Table 3). This is in agreement with the

similar concentrations of milk fat previously reported for cows fed whole flaxseed and those fed ground flaxseed (da Silva et al., 2007). However, feeding ground flaxseed compared to whole flaxseed decreased dry matter content of butter by 6.6% and increased conjugated linoleic acid concentration in butter fat, which concurs with the lower dry matter concentrations that have been reported previously for conjugated linoleic acid enriched butter (Jones et al., 2005). Flaxseed processing increased concentration of polyunsaturated fatty acids in butter fat and higher percentage of unsaturated FA has been positively correlated with better spreadability of butter (Enjalbert et al., 1997). Spreadability is improved with a reduction in milk fat globule size (Goudédranche et al., 2000) as a result of enhanced water retention by the milk fat globule membrane, which, in turn, induces a reduction in butter dry matter content (Michalski et al., 2002).

Thus, higher polyunsaturated fatty acid concentrations in butter with flaxseed processing may decrease milk fat globule size and increase water retention, therefore decreasing dry matter content. Monensin supplementation increased conjugated linoleic acid concentration in butter fat but the effect on polyunsaturated fatty acids concentrations would not be important enough to modify dry matter content. Monensin supplementation had no effect on fat concentration of butter (Table 3) although there is a general decrease in fat concentration of milk when adding monensin to a dairy cow diet (Phipps et al., 2000; Bell et al., 2006). On the other hand, differences in milk fat concentration are not always reflected on butter fat concentration. Lower fat concentration in the milk of cows fed fish oil compared to that of cows fed no fat supplement resulted in similar fat concentrations in butter (Baer et al., 2001), thus suggesting that the effects of diets on fat concentration of milk and that of butter could be different. Supplementation with a source of polyunsaturated fatty acids (e.g. fish meal) results in smaller average fat globule size (Avramis et al., 2003; Jones et al., 2005) and lower milk fat concentration and fat losses in butter while increasing concentration of trans vacenic acid (TVA) and conjugated linoleic acid in milk fat (Baer et al., 2001). Similarly, a decrease in milk fat concentration and an increase in

Table 3 - Characteristics of butter¹

	Experimental diet				SE	Probability		
	Whole flaxseed	Whole flaxseed + monensin	Ground flaxseed	Ground flaxseed + monensin		Flaxseed	Monensin	Flaxseed × monensin
Fat, %	65.6	64.0	69.0	61.7	3.0	0.85	0.15	0.35
Dry matter, %	77.2	78.4	73.8	71.5	1.9	0.01	0.78	0.36

¹Least squares means with pooled standard error (SE).

concentration of trans vacenic acid and conjugated linoleic acid in milk fat with monensin supplementation could contribute in decreasing fat globule size and fat losses in butter and results in similar fat concentrations in butter of cows supplemented or not with monensin although milk fat concentration was different (da Silva et al., 2007).

Conclusions

Feed ground compared to whole flaxseed decreases relative percentages of medium-chain fatty acids and it increases those of *cis9,trans11-18:2*, *cis3-18:3*, and omega 3 fatty acids in butter fat. Monensin supplementation increases relative percentages of *cis9,trans11-18:2*. Both flaxseed processing and monensin supplementation would then improve butter fatty acids profile from a human health perspective. Relative percentages of saturated and medium-chain fatty acids increases with a longer storage period while those of monounsaturated and long-chain fatty acids decreases. These results suggest that butter enriched in polyunsaturated fatty acids could have a shorter shelf life due to the negative effect of storage on fatty acids profile which may result in oxidation and rancidity.

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