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Nutrients balances and milk fatty acid profile of mid lactation dairy cows supplemented with unsaturated fatty acid

Balanço de nutrientes e perfil de ácidos graxos do leite de vacas leiteiras no terço médio de lactação suplementadas com ácidos graxos insaturados

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SUMMARY

The objective was to evaluate the effect of unsaturated fatty acid sources supplementation on nutrients balances and milk fatty acid profile of mid lactation dairy cows. Twelve Brazilian Holstein cows in the mid lactation (mean of 128 days) and (580 ± 20kg of weight; mean ± SD) with milk yield of 25kg/d were assigned randomly into three 4 x 4 Latin square, fed the following diets: control (C); refined soybean oil; (SO); whole soybean raw (WS) and; calcium salts of unsaturated fatty acids (CSFA). Milk yield was 26.6; 26.4; 24.1 and 25.7 to the diets CO, SO, WS and CSFA respectively. Cows fed the WS treatment produced less milk (1.95kg/d of milk), fat and lactose than did cows fed the SO and CSFA. Cows fed the CSFA treatment showed less blood, urine (g/d) concentrations of N more energetic efficiency and intake of energy than did cows fed the SO treatment. Cows fed the unsaturated fatty acids sources showed more C18:2 cis-9, trans-11 CLA and trans-C18:1 FA concentration in milk than did cows fed the CO treatment. Diets with whole soybeans and soybeans oil provide more efficient digestive processes, and increase milk composition of unsaturated fatty acids.

Key words: digestibility, lipids, performance, whole raw soybeans

RESUMO

Objetivou-se avaliar o uso de fontes de ácidos graxos insaturados nas rações de vacas leiteiras sobre o balanço de nutrientes e o perfil de ácidos graxos do leite**.** Foram utilizadas 12 vacas Holandesas no terço médio de lactação (média de 128 dias) e (580 ± 20 kg de peso; média ± DP) com produção média de leite de 25kg/dia distribuídas aleatoriamente em três quadrados latinos balanceados 4 x 4 alimentadas com as seguintes dietas: controle (C); óleo de soja refinado; (OS); grão de soja integral (GS) e; sais de cálcio de ácidos graxos insaturados (SCAG). A produção de leite obtida foi de 26,6; 26,4; 24,1 e 25,7kg/dia para as dietas CO, OS, GS e SCAG respectivamente. As vacas alimentadas com a dieta GS produziram menos leite (1,95kg/dia), gordura e lactose em relação às vacas alimentadas com as dietas OS e SCAG. As vacas alimentadas com a dieta SCFA, apresentaram menores concentrações de nitrogênio uréico no sangue, leite, urina (g/dia), maior eficiência do uso de nitrogênio e consumo de energia em relação a dieta OS. As vacas alimentadas com as dietas contendo fontes de ácidos graxos insaturados demonstraram maiores concentrações no leite dos ácidos graxos C18:2 cis-9, trans-11 e C18:1, trans-11, em relação as vacas alimentadas com a dieta CO. O uso de grão de soja integral e óleo de soja promovem maior eficiência dos processos digestivos e aumentam as concentrações de ácidos graxos insaturados no leite de vacas leiteiras.

Palavras chave: desempenho, digestibilidade, grão de soja integral, lipídios

INTRODUCTION

The effect of supplemental fatty acids on lactation performance may be evaluated by substituting fat for concentrates in a manner that increases net energy liquid content of the test ration compared with a control ration (JENKINS et al., 2007).

Dietary fatty acid serves a number of physiological functions in lactating dairy cows and has effect on the milk fatty acid (FA) profile. Intermediates of FA biohydrogenation are biologically active and modify reproductive efficiency, milk fat synthesis, and metabolism of cows (HAVARTINE & ALLEN, 2006).

Whole oilseed such as soybeans has been utilized in dairy rations to supply additional fat and protein. Although soybeans can be fed raw, extruding or roasting increases the amount of protein escaping ruminaI degradation. Whole raw soybeans (WRS) are commonly used as source of supplemental fat, containing an average of (DM) 190g/kg of DM fat and 392g/kg of DM CP and are an economical and convenient source of dietary fat and protein (NRC, 2001).

Calcium salts of long-chain FA are FA complexed with a calcium ion making them insoluble. Microbes cannot absorb FA as calcium salts and FA salts have little effect on microbial fermentation. However, the complex dissociates as ruminal pH decreases allowing microbial uptake and biohydrogenation (PALMQUIST, 2007). According Glasser et al. (2008) and Allen (2005) although FA supplements are often used to increase energy density of the diet, their efficacy depends on the digestibility of the added FA and their effect on digestibility of other nutrients.

The aim of the present experiment was to quantify the effect of unsaturated fatty acid sources supplementation on nutrients balances and milk fatty acid profile of mid lactation dairy cows

MATERIAL AND METHODS

Twelve Brazilian Holstein cows in the mid lactation (128 day in milk), (580 ± 20kg of weight; mean ± SD) were randomly assigned used in three 4 × 4 Latin's squares design. The experiment was realized in 21 days periods with 14 days of adaptation and 7 days of sample collection.

Treatments were a control diet containing no fatty acids supplements containing 25g of FA/ kg of DM (CO); diet with inclusion of refined soybean oil in 3% in the total dry matter (SO); diet with inclusion whole soybean raw of 16% in the total dry matter with (WS); diet with inclusion of calcium salts of unsaturated fatty acids (CSFA), (Ca Soaps of CSFA, Megalac-E, Química Geral do Nordeste and Arm & Hammer, Inc.).

Diets containing 60% forage (corn silage) and 40% of concentrate (DM basis) were balanced for minerals and vitamins and were formulated to meet the nutrient demand of cows according to the NRC (2001) recommendations. Throughout the experiment, cows were housed in tie stalls and diets were fed as a TMR twice daily at 08:00h and at 13:00h. Amounts of feed offered and orts were weighed for each cow daily and restricted from 5 to 10% of intake on an as-fed basis. Samples of all diet ingredients (0.5kg) and orts from each cow (0.5kg) were collected daily on d 14 to 21 and mixed to representative sample to analysis.

Samples of food provided, orts and feces were analyzed levels for dry matter (DM, AOAC 950.15), ash (AOAC, 942.05), ether extract (EE, AOAC 920.39), crude protein (CP) (AOAC, 984.13), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and Lignin contents according to methods described by AOAC (2006). The crude protein (CP) was obtained by multiplying of the total nitrogen content by 6.25. The total carbohydrate (TC) were calculated by Sniffen et al. (1992), where TC=100−(%CP+%EE+%MM). The levels of non-fiber carbohydrates (NFC) were estimated by HALL (2000) where: NFC=100−[(% CP−% CPurea+% UREA)+%EE+%MM+%NDF]. The total digestible nutrients were calculated according to National Research Council (NRC, 2001), where:
TDN= dNFC + dCP + (dFA 2.25) + dNDF−7, where dCP, dNFC, dNDF and dFA represent the total of this digestible nutrients. The total digestible nutrients TDN=dCP+dNDF+(dEE 2.25)+ dNFC were calculated according to Weiss et al. (1992). The contents of neutral detergent fiber (NDF), and acid detergent fiber (ADF) were obtained according to Mertens et al. (2002), using α-amylase and without addition of sodium sulfite in the NDF determination, at Ankon® System. The diets components, chemical composition and foods profile fatty acids is presented in Tables 1 and 2.

The feces were collected at the 15th to 18th day of each experimental period, always before the morning and afternoon milking, stored in plastic bags and kept at –20°C. At the end of the collection period, samples were composed by each animal based in dry matter. For the determination of total apparent digestibility of dry matter and nutrients, the total quantity of fecal dry matter excreted was estimated by the concentration of internal marker indigestible acid detergent fiber (iADF). To evaluate the indigestible components contents, the processed samples were packed in bags of none-woven textile (CASALI et al., 2008) - (NWT-100g/m2), with dimensions of 4 x 5cm. The aliquots were packed in all bags, according to the ratio of 20mg of dry matter by square centimeter of surface (CASALI et al., 2008). Two Holstein cows were adapted during 7 days with a diet based on soybean meal and ground corn and corn silage, before samples incubation. After the adaptation period, the samples were incubated in the rumen for 240 hours, according to technique described by (CASALI et al., 2008). The bags were removed from rumen, and washed in flowing water until total clearing, and immediately placed in forced ventilation oven (60º/72 hours).

The analysis of the fatty acid profile of foods was realized according to Folch et al. (1957) and the samples of milk according to Feng et al. (2004) and separated fat were methylated and the methyl esters were formed according to Kramer et al. (1997). Fatty acids were quantified by gas chromatography (GC Shimatzu 2010 with automatic injection) using a capillary column SP-2560 (100m × 0.25mm of diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). The initial temperature was 70°C for 4 minutes (13°C/minute) until reach 175 ºC, keeping for 27 minutes. After a further increase of 4°C/minute was started until 215°C, and keeping during 31 minutes. Hydrogen (H_2) was used as carrier gas with a flow of 40cm^3/s.

Table 1. Ingredients, nutrients and fatty acids composition of experimental diets

Item	Treatments[1]			
	C	SO	WS	CSFA
Ingredient	% OF DM			
Corn silage[2]	58.0	58.0	58.0	58.0
Ground corn	21.9	18.9	18.7	18.9
Soybean meal	16.4	16.4	3.6	16.4
Soybean oil	-	3.0	-	-
Whole raw soybean	-	-	16.0	-
Calcium salts of unsaturated fatty acids	-	-	-	3.0
Urea	0.7	0.7	0.7	0.7
Ammonium sulfate	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.6	0.6	0.6	0.6
Magnesium oxide	0.0	0.0	0.0	0.0
Mineral[3]	2.0	2.0	2.0	2.0
Limestone	0.1	0.1	0.1	0.1
Salts	0.2	0.2	0.2	0.2
Nutrients	% of DM			
Dry matter	52.9	53.2	53.2	53.1
Organic matter	91.5	91.6	91.7	90.9
Crude protein	17.8	17.5	17.1	17.5
NDIN[4]	14.9	14.4	16.8	14.4
ADIN[5]	10.8	10.5	10.7	10.5
Ether extract	2.8	5.7	4.9	5.1
Total carbohydrates	71.2	68.5	69.7	68.3
Neutral detergent fiber	41.5	41.0	43.2	41.0
NDFap	38.6	38.2	39.4	38.2
Non-fiber carbohydrates	37.3	35.2	34.1	35.1
NFCap	38.1	36.0	35.8	36.0
Acid detergent fiber	26.9	27.8	26.9	24.8
Lignin	3.3	3.2	4.1	3.2
Mineral matter	7.6	7.6	7.5	7.6
TDN[6]	67.33	70.19	69.12	69.34
NE_L[6] (Mcal/kg of DM)	1.62	1.73	1.73	1.70
Fatty acids	g/100g of FA			
C14:0	0.38	0.37	0.39	0.39
C16:0	15.72	15.68	15.36	15.62
C18:0	3.10	3.12	3.19	3.08
C18:1 *CIS*	12.25	12.50	12.47	12.52
C18:2	30.22	30.02	28.43	30.23
C18:3	2.28	2.23	2.09	2.18
Other	3.09	3.08	2.83	3.24

[1]CO = control; SO = soybean oil; WS = whole raw soybeans; CSFA = calcium salts of unsaturated fatty acids; [2]Corn silage contained 32.3 DM (as fed), and 53.11% NDF, 7.0% CP, 17.3% indigestible NDF, and 7.4% ash on a DM basis; [3]Containing per kilogram: calcium - 120g; phosphorus - 73g; sulfur - 30g; magnesium - 44g; copper - 340mg; zinc - 1.350mg; manganese - 940mg; cobalt - 3mg; iodine - 16mg; selenium - 10mg; iron - 1.064mg; vitamin A - 100.000IU; vitamin D - 40.000IU; vitamin E – 60IU; 3% natural matter; [4]% nitrogen insoluble in neutral detergent (% NT), nitrogen insoluble in acid detergent (%NT).
[5]Estimated by the equations of NRC (2001).

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Table 2. Composition of fatty acids of the ingredients (g/100g of fatty acids)

FA	Ingredients					
	Soybean oil	CSFA	Whole Soybean	Ground corn	Corn silage	Soybean meal
C14:0	0.55	0.14	0.20	0.07	1.20	0.56
C16:0	10.22	8.04	12.97	11.55	18.17	16.21
C18:0	3.77	2.37	3.72	3.24	3.24	3.17
C18:1 *cis*	21.67	22.26	14.27	13.35	12.47	12.78
C18:2	48.90	43.09	36.26	42.81	22.19	48.59
C18:3	4.87	3.35	4.21	6.60	-	5.11
Other	-	5.21	0.16	0.42	4.58	2.11

CSFA = Calcium salts of fatty acids (Megalac-e®)

Four patterns were used during the identification process to identify of fatty acids that are formed during the biohydrogenation of unsaturated fatty acids: standard C4-C24 of fatty acids (Supelco ® TM 37), vacenic acid C18: 1 trans-11 (V038- 1G, Sigma ®), CLA C18: 2 trans-10, cis-12 (UC-61M 100mg), CLA and C18: 2 cis-9, trans-11 (UC-60M 100mg), (NU-CHEK-PREP USA ®).

For the calculation of nitrogen balance, was performed the determination of the creatinine concentration in urine according to methodology described by (RENNÓ et al., 2008). Spot samples of 50 ml of urine were obtained from all cows at the 20th day of each experimental period, four hours after the morning feeding, during urination stimulated by massage in the vulva. A sample of pure urine was stored for determination of total nitrogen compounds, urea and creatinine. Creatinine concentrations were determined using commercial kits (Laborlab®), with kinetic calorimetric enzymatic reaction in equipment SBA-200 CELM®. Total daily urinary volume was estimated dividing daily creatinine urinary excretion by the observed values of the creatinine concentration in urine of the spot samples, according to (CHIZZOTTI et al., 2007). Daily creatinine urinary excretion was estimated from the proposition of 24.05mg/kg of body weight (GONZÁLEZ-RONQUILLO et al., 2003). Then, with the average daily creatinine excretion and the creatinine concentration (mg/dl) in the spot urine sample, the total urine volume was estimated, in liters per cow per day, for calculating nitrogen balance.

The total nitrogen of the urine samples was determined according to methods described by (AOAC, 984.13), where the amount in grams of nitrogen per 100mL of urine was obtained by dividing the crude protein value of the samples by factor 6.25 for urine samples. Nitrogen balance was obtained by subtracting the total nitrogen in grams consumed by the values of nitrogen in urine and feces obtaining in the values of retained nitrogen in grams and in percentage of total nitrogen.

The analysis of the urea concentration in milk deproteinized were performed using commercial kits (Laborlab® and CELM®). The milk urea nitrogen (MUN) concentration was determined indirectly using the following formula: MUN = urea (mg/dl)/2.14. Cows were mechanically milked twice a day, at 06:30h and at 15:30h, being the milk yield recorded daily throughout the experimental period.

Milk yield was corrected for 3.5% of fat (FCM) according to formula, where FCM = (0.432 + 0.1625 * milk fat content) * kg of milk. Samples used for analysis of milk composition were obtained at the 15th and 18th day of each experimental period, each sample coming from the two daily milkings. The contents of fat, protein and lactose were determined for infrared spectroscopy.

The efficiency of energy utilization and energy balance were calculated according Harvatine & Allen, (2006).

Data were analyzed using the PROC MIXED procedure Version 9.1.3 (SAS Institute, 2004) according to the following statistical model:

$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$

Where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow ($_i$ = 1 to 12), P_j = fixed effect of period ($_j$ = 1 to 4), Tk = fixed effect of treatment ($_k$ = 1 to 4), and e_{ijk} = residual error.

Period by treatment interaction was evaluated, but was removed from the statistical model when not significant (P > 0.05). Period by treatment interaction was not significant for any variable of primary interest. Data points with Studentized Residuals greater than 3 were considered outliers and excluded from analysis.

To determine differences between treatments were used orthogonal contrasts: C1= control versus fat sources (soybean oil; whole soybean and calcium salts of fatty acids). The aim this contrast was to evaluate the differences between control diets and unsaturated fatty acid sources supplementation; C2= soybean oil versus calcium salts of fatty acids and whole raw soybean. The aim this contrast was to evaluate the differences between a free fatty acids source or no complexed, and two unsaturated fatty acid sources protected or complexed; C3= whole soybean versus calcium salts of unsaturated fatty acids. The aim this contrast was to evaluate the differences between two unsaturated fatty acid sources protected or complexed. Differences were considered significant for P < 0.05.

RESULTS AND DISCUSSION

Unsaturated fatty acids supplementation of dairy cows diets decreased dry matter intake (P<0.05; Table 3). It was observed reduction of 6.91% when compared to control diet for the unsaturated fatty acids diets. Cows fed the whole soybeans treatment produced less milk (1.95kg/d of milk), fat and lactose than did cows fed the SO and calcium salts of fatty acids (Table 3). There were no difference among the treatments to protein and lactose in milk contents in milk.

Milk production corrected did not differ between cows fed the control and unsaturated fatty acids treatments. Cows fed the calcium salts of fatty acids treatment had less concentrations fat than did cows fed the soybeans oil treatment Freitas Junior et al., 2010). Physiologically, the FA CLA C18:2 trans-10, cis-12, according to Peterson et al., (2003), cause changes that can be attributed as being mainly due to the reduction of fatty acids produced by synthesis of new in the mammary gland. Although this theory was already accepted by the scientific community, data of recent studies have shown that this process presents little more complex. According to Peterson et al. (2003) the reduction of milk fat can be attributed to a series of physiological actions: coordinated decrease in the abundance of mRNA of several genes of enzymes linked to synthesis of new of fatty acids (acetyl CoA carboxylase, fatty acid synthase); consumption and transport of circulating AG (lipoprotein lipase, FA binding protein); desaturation of fatty acids (Δ9 - desaturase), and the synthesis of triglycerides (fatty acyl CoA ligase, glycerol phosphate acyl trasferase and acyl glycerol phosphate acyl transferase).

Table 3. Intake, milk yield and composition for mid lactation dairy cows

Item	Treatments[1]				SEM[2]	P[3]		
	C	SO	WS	CSFA		C1	C2	C3
kg/day								
Dry matter intake	17.73	16.84	16.68	15.99	0.32	0.009	0.420	0.041
Milk	26.62	26.37	24.13	25.70	0.79	0.057	0.007	0.380
3,5% FCM	24.50	24.41	23.13	23.25	2.74	0.065	0.980	0.854
Milk fat	0.81	0.78	0.78	0.70	0.04	0.311	0.003	0.403
Mik protein	0.75	0.74	0.68	0.71	0.02	0.043	0.046	0.200
Milk lactose	1.18	1.17	1.08	1.15	0.03	0.203	0.029	0.692
Milk composition (%)								
Fat	3.07	2.96	3.35	2.83	0.02	0.119	0.022	0.087
Protein	2.82	2.82	2.84	2.78	0.02	0.728	0.232	0.374
Lactose	4.53	4.52	4.53	4.50	0.02	0.840	0.776	0.805
MUN (mg/dL)	8.80	8.57	8.14	7.20	0.46	0.077	0.598	0.020

[1]C =control; SO=soybean oil; WS=whole raw soybeans; CSFA=calcium salts of fatty acids (Megalac-E®); [2]SEM = standard error of the mean. Value of Probability to: C1 = control vs fat sources (soybean oil, whole raw soybean and calcium salts of fatty acids), C2 = whole raw soybean vs calcium salts of fatty acids and soybean oil; C3 = soybean oil vs calcium salts of fatty acids.

In this study there was no effect (P>0.05) of using different fatty acids sources on the contents of milk non-protein nitrogen, true protein, casein, casein/true protein ratio, whey protein, and on all these fractions expressed as percentage of crude protein as reported by Freitas Junior et al. (2010). According to Rabiee et al. (2012), in meta-analysis of 200 papers the inclusion of fat in the diets for dairy cows had marked effects on milk, milk fat, production, DMI, and content of fat and protein in milk, with the lower DMI combined with higher milk and milk fat production showed that fats could improve the efficiency of milk production. In this study there was not increase of productive performance, but there was increase of efficiency of use of energy to cows feed with fatty acids sources. Moallem et al. (2007) observed lower intake for cows supplemented with calcium salts of fatty acids in relation to other fat sources. According to the NRC (2001), the addition of calcium salts of fatty acids in diets of dairy cows results in linear decrease of dry matter intake. However, in some studies occurred increase of the intake (ALLEN, 2005). These results can be attributed to the low heat increment of fat during periods of heat stress, or to the reduction of propionate production when fat sources substituted grains in the diets (NRC, 2001). In this study there were not effects of fatty acids sources in diets on ruminal fermentation. However, cows fed calcium salts of fatty acids showed less dry matter intake. Differences in acceptability in this study contributed to differences in dry matter intake. Fat supplements were found to vary in acceptability with lower acceptability for the calcium salts of fatty acids, than for tallow, sodium alginate encapsulated dry tallow, or prilled long chain fatty acids were feed in diets of dairy cows (ALLEN, 2005).

Cows fed the soybeans oil, whole soybeans and calcium salts of fatty acids diets had less nitrogen intake (P<0.007) and fecal excretion of nitrogen (P<0.001) - (N, g/d total of nitrogen) - (Table 4). However, there were not effects diets on excretion of nitrogen urine and milk. Cows fed the calcium salts of fatty acids treatment had less BUM, MUN, and MUN (g/d) concentrations of N than did cows fed the soybeans oil treatment (P<0.04).

Table 4. Nitrogen balance and daily nitrogen excretion in urine and milk

Item	Treatments[1]				SEM[2]	P[3]		
	C[a]	SO[b]	WS[c]	CSFA[d]		C1	C2	C3
N g/day								
Intake	507.80	482.52	476.67	460.21	9.55	0.007	0.576	0.050
Feces	159.00	135.58	141.84	123.89	4.78	0.001	0.128	0.200
Urine	186.32	192.99	164.25	173.48	7.91	0.573	0.289	0.344
Milk	125.51	98.82	116.30	115.12	5.30	0.199	0.459	0.266
Balance	43.11	37.06	50.01	44.45	9.94	0.970	0.658	0.760
% N total								
Feces	31.13	27.42	30.64	27.37	0.73	0.065	0.036	0.977
Urine	38.22	40.02	39.95	38.32	1.80	0.894	0.262	0.693
Milk	23.82	25.25	24.34	25.14	0.49	0.153	0.280	0.900
Balance	7.00	7.73	9.92	8.78	1.98	0.665	0.705	0.836
Efficiency[4]n	0.236	0.248	0.243	0.243	0.04	0.247	0.749	0.605
mg/dL								
BUN[5]	17.41	17.95	19.81	13.24	0.69	0.718	0.001	0.002
MUN[6]	8.80	8.57	8.14	7.20	0.46	0.077	0.598	0.020
Daily excretion								
MN[7] (g)	11.96	11.79	10.93	11.34	0.32	0.044	0.046	0.202
MUN (g)	4.63	4.81	4.83	3.35	0.23	0.362	0.034	0.009
NU[8](g)	179.90	157.13	174.68	181.90	7.48	0.617	0.778	0.249
UNU[L](g)	167.99	102.95	167.99	170.37	13.27	0.886	0.303	0.061
UN[9] (mg/kgBW)	332.18	283.15	326.62	351.88	14.78	0.706	0.778	0.084
NUU[10](g/kgBW)	279.03	186.88	314.43	332.64	26.35	0.984	0.342	0.035

[1]C =control; SO=soybean oil; WS=whole raw soybeans; CSFA=calcium salts of fatty acids (Megalac-E®); [2]SEM = standard error of the mean; [3]Value of Probability to: C1 = control vs fat sources (soybean oil, whole raw soybean and calcium salts of fatty acids), C2 = whole raw soybean vs calcium salts of fatty acids and soybean oil; C3 = soybean oil vs calcium salts of fatty acids; [4]kg N in milk/kg N intake; [5]blood urea nitrogen; [6]milk urea nitrogen milk nitrogen; [7]nitrogen urine; [8]urea nitrogen urine; [9]urine nitrogen;[10]nitrogen urea urine.

The difference of intake and fecal excretion nitrogen of animals in control diet can be partially explained by the variation on dry matter intake when compared to the fatty acids added diets and control diet (Table 3).

Moreover alterations were observed in nitrogenous compounds intake and on fecal nitrogen excretion when data was analyzed quantitatively in grams for day (Table 4). Spanghero & Kowalski (1997) evaluated 35 experiments and 135 different diets, with average dry matter intake of 17.6kg/day and average milk yield of 26.1kg/day, and reported 39 g/day as the average value for nitrogen balance for dairy cows. At the present study, the average nitrogen balance was 43 g/day, similar to the value reported by Spanghero & Kowalski (1997). Gozho & Mutsvangwa, (2008) evaluated diets supplemented with CSFA, linseed or micronized soy for cows at the beginning of lactation (8% of ether extract) and did not observe differences in nitrogen balance among the sources used, which was similar to the results presented by the present study (9.5% of total nitrogen intake).

Cows fed the soybeans oil, whole soybeans and calcium salts of fatty acids treatments had less intake of gross and digestible energy intake than did cows fed the control treatment (P<0.01) (Table 5). However, there was no difference to EBW change, NE_L and empty BW gain among the treatments (NRC, 2001) - (P<0.03) - (Table 5).

Table 5. Fat source effects on efficiency of energy utilization and energy balance

Item	Treatments[1]				SEM[2]	P[3]		
	C	SO	WS	CSFA		C1	C2	C3
Intake								
Gross energy[4] Mcal/d	75.51	74.33	73.60	61.37	1.59	0.002	0.003	0.001
DE,[5] Mcal/d	49.40	50.38	48.80	42.53	0.67	0.001	0.008	0.001
NEL,[6] Mcal/d	28.23	28.39	28.27	27.04	0.58	0.702	0.536	0.037
Production								
Milk NEL [7], Mcal/d	16.87	16.44	15.70	15.55	0.45	0.045	0.549	0.130
EBW Change, kg /d	0.35	0.24	0.36	0.26	0.04	0.481	0.250	0.883
NEL [8] Empty BW Gain,/d	2.77	3.61	4.05	2.95	0.54	0.288	0.314	0.452
BCS Change, /21 d	0.04	0.04	0.08	0.04	0.03	0.632	0.182	1.000
Balance								
NEL A. Maint,[9] Mcal/d	8.99	9.05	8.92	8.86	0.10	0.511	0.702	0.035
Efficiency								
NEL Milk/DE Intake[10]	0.40	0.40	0.41	0.43	0.01	0.338	0.525	0.019
NEL Prod/DE Intake[11]	0.34	0.32	0.32	0.37	0.08	0.732	0.057	0.003

[1]C =control; SO=soybean oil; WS=whole raw soybeans; CSFA=calcium salts of fatty acids (Megalac-E®); [2]SEM = standard error of the mean; [3]Value of Probability to: C1 = control vs fat sources (soybean oil, whole raw soybean and calcium salts of fatty acids), C2 = whole raw soybean vs calcium salts of fatty acids and soybean oil; C3 = soybean oil vs calcium salts of fatty acids; [4]Obtained by calorimetry bomb; [5]Digestible Energy(intake) (DE) = ((NFCdig X NFC/100)x4.2) + ((NDFdig X NDF/100) X 4.2) + ((CPdig X CP/100) X 5.56) + ((EEdig x EE/100) X 9.4) – 0.3 (NRC, 2001); [6]Net Energy Lactaton(intake) (NEL) = 0.703 X (ME) - 0.19 + ([(0.097 X (ME)+ 0.19)/97] X [EE - 3]) (NRC, 2001); [7]NetEnergy Lactation (milk) = Milk yield (kg) × (0.0929 × fat% + 0.0563 × true protein% + 0.0395 × lactose%)(NRC, 2001); [8]Net Energy gain (empty body weight change) calculated according to NRC (2001); [9]NEL Available for Mantence = NEL(intake) − NEL(BW Gain) − NEL(milk); [10](NEL Milk yield + NEL BW gain)/DE intake; [11](NEL Milk yield)/DE intake.*Model analyzed.

Cows fed the calcium salts of fatty acids treatment had more efficiency of NEL milk/DE intake and NE_L prod/DE intake than did cows fed the soybeans oil treatment (P<0.01). This can be attributed the less intake NEL and digestible energy intake of the cows fed the calcium salts of fatty acids.

This decrease observed in gross energy intake caused by fat supplementation may have resulted from the lower dry matter intake obtained for the animals (Table 3). However, the fatty acid sources have promoted lower (P<0.05) digestible energy intake (Table 5) compared to the control diet.

It is necessary to consider that the composition of body weight gain was not determined, thus, possibly the calculation of the net energy gain of body weight could be overestimating the retained net energy. Besides, the energy balance can be estimated by the calculation of energy conservation, so that there was no difference (P>0.05) on efficiency of energy utilization for maintenance among experimental diets (Table 6).

This model of calculation was chosen for being the most frequently used for calculating energy balance, and for eliminating the errors of increase in live weight gain that could overestimate the increase on maintenance requirements (HARVATINE & ALLEN, 2006). The metabolic live weight can be used to predict the energy requirements of maintenance among animals differing on live weight, but cannot be used to estimate changes on net energy maintenance requirements with live weight gain, because there are variation on body composition of the studied animals (NRC, 2001).

Cows fed the soybeans oil, whole soybeans and calcium salts of fatty acids treatments had less concentration of fatty acids C6-C18, <C16, C16, saturated and unsaturated in milk (Table 6). Cows fed the unsaturated fatty acids treatment had more concentration (P<0.01), C18:1cis (P<0.01), C18:3 (P<0.01) and consequently increases concentration of total unsaturated, ration Uns/Satu, and total C18 saturated and unsaturated (P<0.01), than did cows fed the control treatment.

However the cows fed the soybeans oil, whole soybeans and calcium salts of fatty acids treatment showed more C18:2 cis-9, trans-11 CLA and trans-C18:1 FA concentration than did cows fed the control treatment. Cows fed the soybeans oil, and calcium salts of fatty acids treatment had higher concentration of fatty acids 18:2 cis-9, trans-11 CLA and trans-C18:1 FA concentration than did cows fed the WS treatment.

The decrease in concentrations of fatty acids C6-C18, in total fatty acid <C16, and yield of C18 can be attributed to the elevation cis-9, trans-11, and trans-10, cis-12, CLA milk concentrations, when cows were supplemented with fat sources, wide can decrease concentrations of other fatty acids in milk (HAVERTINE & ALLEN, 2006; PALMQUIST, 2007). This result indicates that diets containing all additional fat sources here was level at some incomplete rumen biohydrogenation, which could have caused reduction of the Δ^9 desaturase enzyme activity (CÔRTES et al., 2010), modifying the process of fatty acids synthesis on the mammary gland.

In general, the consequence of changing the fatty acids profile of the fat supplemented diets, is the reduction in the secretion of fatty acids with less than 16 carbons (<16 C). Consequently, milk fat composition was altered, promoting a correspondent increase in most fatty acids (>16 C). Similar results

have also been observed in several studies (PETERSON et al., 2003; LOOR & HERBEIN 2003; MACKLE et al., 2003).
Theoretically, the increase in the proportion of unsaturated fatty acids in the diet would reduce the proportion of unsaturated/saturated C18 fatty acids in milk fat, but can also increase the concentration of trans-C18:1 as a result of biohydrogenation. In this study, this was caused by the use of SO. However, from all experimental diets, the one containing calcium salts of fatty acids had the higher concentration of trans-C18:1.

Table 6. Milk fatty acids profile according to the experimental diets

Item	Treatments[1]				SEM[2]	P[3]		
	C	SO	WS	CSFA		C1	C2	C3
FA, g/100g total FA								
4:0	1.51	1.38	1.48	1.55	0.03	0.565	0.895	0.061
6:0	1.53	1.18	1.38	1.17	0.03	<0.001	0.006	0.873
8:0	0.99	0.72	0.85	0.67	0.07	<0.001	0.004	0.223
10:0	2.36	1.64	1.85	1.44	0.08	<0.001	0.002	0.068
12:0	2.81	1.97	2.11	1.75	0.25	<0.001	0.020	0.085
14:0	10.76	8.29	8.73	7.52	0.02	<0.001	0.016	0.048
15:0	0.97	0.77	0.78	0.78	0.44	<0.001	0.539	0.753
16:0	30.76	25.26	26.79	25.52	0.04	<0.001	0.021	0.693
16:1 *cis*	2.01	1.76	1.69	1.73	0.01	<0.001	0.109	0.362
17:0	0.60	0.47	0.48	0.56	0.36	<0.001	0.080	0.003
18:0	12.10	15.53	15.74	14.86	0.11	<0.001	0.321	0.293
18:1 *trans*	0.32	0.48	0.16	0.99	0.69	0.326	0.023	0.069
18:2 n-6*, trans*	0.21	0.24	0.15	0.29	0.01	0.292	<0.001	0.029
18:2 n-6, *cis*	2.10	2.27	2.69	3.00	0.07	<0.001	0.639	<0.001
18:1*cis* 9	3.10	3.47	1.38	5.77	0.02	0.766	0.047	0.211
18:3	0.26	0.19	0.29	0.29	0.01	0.958	0.325	0.092
20:0	0.11	0.14	0.12	0.12	0.05	0.228	0.647	0.350
cis-9,*trans*-11 CLA	0.41	0.95	0.44	0.79	0.00	0.005	<0.001	0.126
trans-10,*cis*-12 CLA	0.01	0.02	0.02	0.02	0.03	0.003	<0.001	<0.001
Total								
<C16	23.28	17.71	19.09	16.55	0.51	<0.001	0.003	0.113
C16	44.08	30.83	29.94	28.21	1.70	<0.001	0.898	0.490
>C16	34.47	37.66	36.42	39.87	1.10	0.130	0.332	0.429
Unsat. C18	22.82	24.67	23.41	27.12	0.80	0.161	0.144	0.210
Satu. C18	10.10	11.42	11.75	11.01	0.33	0.051	0.428	0.604
Unsat./Sat. C18	2.29	2.17	2.02	2.46	0.05	0.234	<0.001	<0.001
Saturades	61.84	56.00	57.72	52.77	1.10	<0.001	0.009	0.021
Unsatured	31.11	36.95	33.36	39.22	0.62	<0.001	<0.001	0.010
Uns/Sat.	2.04	1.53	1.76	1.39	0.04	<0.001	<0.001	0.027

[1]C =control; SO=soybean oil; WS=whole raw soybeans; CSFA=calcium salts of fatty acids (Megalac-E®); [2]SEM = standard error of the mean. [3]Value of Probability to: C1 = control vs fat sources (soybean oil, whole raw soybean and calcium salts of fatty acids), C2 = whole raw soybean vs calcium salts of fatty acids and soybean oil; C3 = soybean oil vs calcium salts of fatty acids.

Thus, the determination of the trans-C18:1 in milk fat was essential for the conclusion that there was higher biohydrogenation of this fat source in the rumen.
Diets with whole soybeans and soybeans oil provide more efficient digestive processes, and increase milk composition of unsaturated fatty acids.

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