ADDITION OF UNSATURATED FATTY ACIDS IMPROVES DIGESTION OF MID LACTATING DAIRY COWS

ADIÇÃO DE ÁCIDOS GRAXOS INSATURADOS MELHORA OS PROCESSOS DIGESTIVOS DE VACAS LEITEIRAS NO TERÇO MÉDIO DE LACTAÇÃO

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ADDITIONAL KEYWORDS

Intake. Digestibility. Lipids. Whole raw soybeans.

PALAVRAS CHAVE ADICIONAIS

Consumo. Digestibilidade. Lipídeos. Grão soja cru.

SUMMARY

The objective of the present experiment was to evaluate the effect of different unsaturated fatty acids supplementation sources on digestive metabolism, including nutrient intake and total tract digestibility, microbial protein synthesis and ruminal fermentation in lactating dairy cows. Twelve Holstein mid lactating cows (580±20 kg of body weight; mean±SD, with average of 128 days in milk, and milk yield of 25 kg/d), were assigned randomly into three 4x4 Latin squares, fed the following diets: 1) control (CO); 2) refined soybean oil (SO); 3) whole raw soybean (WS) and; 4) calcium salts of unsaturated fatty acids (CSFA). Milk yields were 26.6; 26.4; 24.1 and 25.7 for cows fed CO, SO, WS and CSFA, respectively. Cows fed SO, WS and CSFA had lesser intake of DM, OM, CP, EE, TC, NFC and TDN than cows fed CO. Animals fed unsaturated fatty acids supplementation had higher DM and EE digestibility, when compared to cows fed CO. Fatty acids supplementation increased ruminal pH and decreased NH_3-N concentrations, when compared to animals fed CO. Daily allantoin excretion, uric acid, milk allantoin, total purine derivatives, absorbed purines, microbial nitrogen, and microbial efficiency did not differ among cows fed different experimental diets. Diets with whole raw soybeans and soybean oil improved digestive metabolism and increased the concentrations of unsaturated fatty acids in milk.

RESUMO

Objetivou-se neste estudo 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 kg de peso corpóreo) com produção média de leite de 25 kg/dia distribuídas aleatoriamente em três quadrados latinos balanceados 4x4 alimentadas com as seguintes dietas: 1) controle (CO); 2) óleo de soja refinado; (SO); 3) grão de soja integral (WS) e; 4) sais de cálcio de ácidos graxos insaturados (CSFA). A produção de leite obtida foi de 26,6; 26,4; 24,1 e 25,7 kg/dia para as dietas CO, SO, WS e CSFA respectivamente. As vacas alimentadas com as dietas OS, GS e SCAG apresentaram menores consumos diários de MS, MO, PB, EE, CT,

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CNF e NDT em relação às vacas do tratamento CO. Os animais alimentados com as dietas contendo ácidos graxos apresentaram maiores valores de digestibilidade da MS e EE em relação a dieta CO. A suplementação de ácidos graxos aumentou os valores de pH e as concentrações de N-NH_3 no rúmen. As excreções de alantoína, ácido úrico, alatoína do leite, derivados totais de purinas, total de purinas absorvidas, nitrogênio microbiano, e eficiência microbiana não diferiram entre as dietas experimentais. O uso de ácidos graxos insaturados na dieta de vacas leiteiras altera a eficiência digestiva da dieta dependendo da forma suplementada.

INTRODUCTION

Dietary fatty acid serves a number of physiological functions in lactating dairy cows and has a large effect on the digestive metabolism. Intermediates of fatty acids (FA) biohydrogenation are biologically active and modify reproductive efficiency, milk fat synthesis, ruminal fermentation, nutrient intake and digestibility of cows (Havartine and Allen, 2006).

Commonly used fatty acids sources include oilseeds, such as whole cottonseed, and full-fat soybeans, animal fats, palm oils, and various modifications to these designed to reduce availability of nutrients to biohydrogenation in the rumen (Rabiee *et al*., 2012).

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 ruminal degradation. Whole raw soybeans (WS) are commonly used source of supplemental fat, containing an average of 190 g/ kg of fat and 392 g/ kg of crude protein (DM basis) and are an economical and convenient source of dietary fat and protein (NRC, 2001).

Calcium salts of long-chain FA are FA bonded with calcium ions, making them insoluble. Microbes cannot absorb FA in the form of calcium salts, and CSFA have little effects on microbial fermentation. However, the complex dissociates as ruminal pH decreases allowing microbial uptake and biohydrogenation of FA (Wu and Palmquist, 1991). According to Allen *et al.* (2005), despite of FA supplements often increases diet energy density, their efficacy depends on both digestibility of added FA and effect on digestibility of other nutrients. Rabiee *et al*. (2012) reported in a meta-analysis and meta-regression that fatty acids addition in diets of dairy cattle had marked effects on milk yield, milk fat production, DMI, and fat and protein milk content.

The objective of the present study was to evaluate the effect of the supplementation of unsaturated fatty acids on digestive metabolism, nutrient intake and total tract digestibility, microbial protein synthesis and ruminal fermentation in lactating dairy cows.

MATERIAL AND METHODS

Twelve Holstein cows in mid lactation (128 days in milk, 580 ± 20 kg of body weight; mean ± SD) were randomly assigned to three 4×4 Latin's squares design. The experiment consisted of 21d periods with 14d of adaptation and 7d of collection.

Dietary treatments were formulated according to NRC (2001) as follows: a control diet (CO); soybean oil (SO, with 3 % refined soybean oil inclusion on diet DM basis); whole raw soybean (WS, with 16 % whole raw soybean inclusion on diet DM basis) and; calcium salts of unsaturated fatty acids (CSFA, with 3 % calcium salts of unsaturated fatty acids inclusion on diet DM basis, Megalac-E, Química Geral do Nordeste and Arm & Hammer, Inc.) **(table I)**.

Experimental diets contained 58 % forage (58:42, corn silage:concentrate), whole raw soybeans, dry ground corn, soybean meal, a mineral and vitamin mix. Throughout the experiment, cows were housed in tie stalls and diets were fed as a total mixed ration (TMR), twice daily at 08:00 h and 13:00 h. Amounts of feed offered and orts were

***Table I**. Ingredients, nutrients and fatty acids composition of experimental diets.* (Ingredientes, nutrientes e perfil de ácidos graxos das dietas experimentais).

	Control	Soybean oil	Whole raw soybeans	Calcium salts of unsaturated fatty acids
Corn silage[1]	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[2]	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 Neutral detergent insoluble N (% NT)	14.9	14.4	16.8	14.4
ADIN Acid detergent insoluble N (% NT)	10.8	10.5	10.7	10.5
Ether extract	2.44	4.76	4.26	4.79
Total carbohydrates	71.2	68.5	69.7	68.3
Neutral detergent fiber (NDF)	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 (ADF)	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[3]	67.33	70.19	69.12	69.34
NE_L[3] (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	0.38
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]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.
[2]Containing per kg: Ca: 120 g; P: 73 g; S: 30 g; Mg: 44 g; Cu: 340 mg; Zn: 1350 mg; Mn: 940 mg; Co: 3 mg; I: 16 mg; Se: 10 mg; Fe: 1064 mg; Vit A: 100.000 IU; Vit D: 40.000 IU; Vit E: 60 IU.
[3]Estimated by the equations of NRC (2001).

weighed for each cow daily and orts were restricted to 5 to 10 % of intake on an as-fed basis. Samples of all diet ingredients (0.5 kg) and orts from each cow (0.5 kg) were collected daily on d 14 to 21 and combined into 1 sample to bromatological analysis. Milk yield was corrected for 3.5 % of fat (FCM) according to the formula:

FCM = (0.432 + 0.1625 * milk fat content) * kg of milk

Levels of 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 (sa) were analysed in the feed offered, orts and feces samples, according to the methods described by AOAC (2006).

The total carbohydrates (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 (1998) where:

NFC=100"[(% CP»% CPurea+% UREA)+%EE+ %MM+%NDF]

The total digestible nutrients were calculated according to NRC (2001), where:

TDN=dNFC+dCP+(dFA 2.25)+dNDF–7

dCP, dNFC, dNDF and dFA representing the total of those digestible nutrients.

The total digestible nutrients were calculated according to Weiss *et al*. (1992), as follows:

TDN=dCP+dNDF+(dEE 2.25)+ dNFC

The contents of neutral detergent fiber (NDF), and acid detergent fiber (ADF) were obtained according to method described by Mertens *et al*. (2002), using α-amylase and without addition of sodium sulfite in the NDF determination (TE-149 fiber analyzer, Tecnal Equipaments for Laboratory Inc., Piracicaba. Brazil).

Feces samples were collected from day 15 to 18 of each experimental period, before milkings, stored in plastic bags and kept at –20ºC. Samples were composed by each animal, based in dry matter, at the end of the collection period. For the determination of total tract digestibility of dry matter and nutrients, the total fecal dry matter excretion was estimated by the concentration of indigestible acid detergent fiber (iADF). To evaluate the contents of indigestible components, the processed samples were packed in bags of none-woven textile (NWT-100g/m^2), with dimensions of 4 x 5cm. The aliquots were packed in all the bags, according to the ratio of 20 mg of dry matter by square centimeter of surface (Casali *et al*., 2008). Before samples incubation, two Holstein cows were adapted for 7 days with a diet based on soybean meal and ground corn, and receiving corn silage as forage. After the adaptation period, the samples were incubated in their rumen for 240 hours, according to the technique described by (Casali *et al*., 2008). After removal from the rumen, the bags were washed in flowing water until total clearing, and immediately placed in a forced ventilation oven (60 ºC/72 hours).

Samples of rumen fluid were collected by esophageal gavage three hours after the morning feeding. Immediately after collection, rumen pH values were determined using a potentiometer. The samples were temporarily placed on ice and then processed for ammonia nitrogen (NH_3-N) and short chain fatty acids (acids acetic, propionic and butyric) determination. The rumen fluid collected was centrifuged at 2000 x g for 15 min, and 2 mL of the supernatant was pipetted and stored in trial tubes containing 1 mL of 1 N sulfuric acid (for later determination of ammonia nitrogen (NH_3-N) concentration), and 1 mL in tubes containing 0.4 mL of formic acid (for determination of

short chain fatty acids). The analysis of the ammonia nitrogen (NH_3-N) concentration was determined by the method with salicylic acid (Verdouw, and Van Echteld, 1978). The ruminal concentration of short chain fatty acids was analyzed using gas chromatography and glass column of 2 m length of 1/83 , packaged with 80/120 Carbopack B-DA/4 % Carbowax 20M (Erwin *et al*., 1961).

For the microbial protein synthesis calculation, the determination of the creatinine concentration in urine was performed according to the 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, by vulva massage stimulation. A sample of pure urine was stored for creatinine level measurements. Creatinine concentrations were determined with commercial kits (Laborlab®), using kinetic calorimetric reaction in automatic biochemical system analyzer (SBA- 200 CELM®). Total daily urinary volume was estimated dividing daily urinary excretion of creatinine by the observed values of the creatinine concentration in urine of the spot samples, according to Chizzotti *et al*. (2007). Daily urinary excretion of creatinine was estimated from the proposition of 24.05mg/kg of body weight (González-Ronquillo *et al*., 2003). Therefore, 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, to calculating nitrogen balance.

The concentration of allantoin and uric acid, in urine and milk were determined by colorimetry method, according to methodology described by Chen and Gomes (1992). Total excretion of purine derivatives, in mmol/ day, was calculated from the sum of amounts of allantoin and uric acid excreted in urine and milk (Orellana Boero *et al*., 2001).

Data were analyzed using the PROC MIXED procedure Version 9.1.3 (SAS, 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);
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 raw soybean and calcium salts of fatty acids). The aim of 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

Fatty acids supplementation did not affect milk yield (MY) and body condition score (BCS) ($p>0.05$, **table II**). However,

***Table II**. Productive performance.* (Desempenho produtivo).

	Treatments				Mean	SEM	p		
	C	SO	WS	CSFA			C1	C2	C3
MY (kg/d)	26.62	26.37	24.13	25.70	25.7	0.79	0.057	0.007	0.380
FCM 3.5 % (kg/d)	24.50	24.41	23.13	23.25	23.8	2.74	0.065	0.980	0.854
BW (kg)	543.5	548.0	535.9	531.8	539.8	6.2	0.387	0.502	0.024
BCS	2.73	2.75	2.73	2.73	2.73	0.12	0.859	0.824	0.676

C= control; SO= soybean oil; WS= whole raw soybeans; CSFA= calcium salts of fatty acids (Megalac-E®); MY= milk yield; FCM= fat corrected milk; BW= Body weight; BCS=body condition score.
SEM= standard error of the mean; p= 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.

cows fed WS presented lesser FCM (1.2 kg/d) than cows fed SO and CSFA **(table II)**. In the present study, there was effect of diets on fat milk content ($p<0.05$). Calcium salts fatty acids diet decreased milk fat content compared with the SO diet (Freitas Junior *et al*., 2010). This result is explained by lesser FCM presented by cows fed WS.

Cows fed fat supplemented treatments had lesser intake of DM, OM, CP, EE, TC, NFC and TDN than cows fed CO **(table III)**. Was observed reduction of 6.91 % on dry matter intake when was compared cows fed CO with cows fed unsaturated fatty acids diets. Cows fed SO had higher dry matter intake than cows fed CSFA **(table III)**. Fatty acids supplementation of dairy cows diets may decrease dry matter and nutrients intake, and it is dependent of the source and type of the supplement used. Generally, animals from the groups receiving FA supplementation had lower dry matter, OM, CP, EE, TC, NDF, NFC and TDN intake, when compared to animals receiving the control diet ($p<0.05$, **table III**). On the other hand, the group supplemented with soybean oil had higher EE intake than the groups receiving other fat sources (WS and CSFA) ($p<0.05$, **table III**).

Despite the fact of fat supplemented diets provided lower TDN intake than control ($p<0.05$), there was no difference in net energy intake among cows fed the experimental diets ($p>0.05$; **table III**).

Cows fed the soybeans oil, whole soybeans and calcium salts of fatty acids treatments had higher DM and EE apparent total tract digestion than cows fed the control treatment. However, animals fed the unsaturated fatty acids supplementation had higher total DM and EE digestibility, when compared to control diet ($p<0.05$, **table III**). Cows fed SO and CSFA had higher DM, OM, CP, EE, NFC and TDN digestibility than cows fed WS ($p<0.05$, **table III**).

The differences in nutrients intake observed between supplemented fat and control diets resulted from the chemical composition of these diets and from the decrease in dry matter intake, which was greater for cows fed CSFA. Allen (2000) evaluated several studies using different fat sources in lactating cows diets, and observed that diets containing calcium salts of fatty acids determined greater decrease of DM intake compared to other fat sources, like free oils, oilseeds and animal fat (Moallem *et al*., 2007). According to the NRC (2001), generally, the addition of calcium salts of fatty acids in diets of dairy cows results in linear decrease of dry matter intake.

When different fat sources are evaluated,

different responses are expected according to the type and level of inclusion of the fat supplement in the diet. For diets containing 5 to 6 % of ether extract (diet DM basis) according to NRC (2001), the addition of oilseeds and partially hydrogenated FA reduces feed intake. The addition of tallow, yellow fat and calcium salts of fatty acids also results in negative effect with linear decrease of DMI. However, change in feed intake, followed by decrease in milk fat content and reduction of ruminal digestion of fiber, are indicators that ruminal fermentation was altered with the addition of fat in the diet, what can probably be justified by the higher energy density and the higher digestibility of the lipid fraction **(table III)** for these diets.

Although, there are many studies about the effects of fat supplementation for lactating cows, the mechanisms by which this supplementation influences intake are not yet fully elucidated, but there are strong evidences that fat effect rumen fermentation, intestinal motility, acceptability of diet, release of intestinal hormones, regulatory

Table III. *Nutrients intake and total apparent digestibility.* (Consumo de nutrientes e digestibilidade aparente total).

	Treatments				Mean	SEM	p		
	C	SO	WS	CSFA			C1	C2	C3
Intake kg/day									
DM (Dry matter)	17.73	16.84	16.68	15.99	16.81	0.32	0.009	0.420	0.041
OM (Organic matter)	16.30	15.15	15.32	14.74	15.46	0.39	0.002	0.478	0.024
CP (Crude protein)	3.22	3.01	2.92	2.91	3.01	0.05	<0.001	0.537	0.111
EE (Ether extract)	0.51	0.98	0.83	0.84	0.79	0.14	<0.001	<0.001	<0.001
TC(Total carbohydrates)	12.83	11.75	11.82	11.19	11.90	0.24	<0.001	0.112	0.032
NDF	7.26	6.78	7.13	6.46	6.91	0.14	0.002	0.003	0.079
NFC	7.04	6.36	6.03	6.07	6.38	0.01	<0.001	0.148	0.032
TDN	12.17	12.10	11.68	11.38	11.83	0.23	0.046	0.845	0.014
Apparent total tract digested (%)									
DM	66.26	69.20	66.90	69.81	68.04	0.89	0.048	0.042	0.650
OM	65.59	67.24	64.24	65.69	65.69	0.48	0.876	0.021	0.149
CP	69.34	72.58	68.56	72.75	70.81	0.75	0.152	0.007	0.919
EE	80.13	90.88	85.60	88.89	86.37	0.81	<0.001	0.001	0.160
TC	64.73	64.03	61.84	63.53	63.53	0.56	0.140	0.096	0.701
NDF	48.80	49.32	50.59	50.30	49.75	0.56	0.244	0.494	0.461
NFC	74.45	74.60	67.92	72.28	72.31	0.90	0.072	0.002	0.226
TDN[1]	67.46	69.41	66.12	68.91	67.97	0.53	0.508	0.010	0.699
Body weight (%)									
DMI (Dry matter intake)	3.28	3.11	3.11	3.08	3.13	0.054	0.006	0.585	0.053
NDF	1.33	1.24	1.32	1.19	1.27	0.024	0.008	0.001	0.223

C= control; SO= soybean oil; WS= whole raw soybeans; CSFA= calcium salts of fatty acids (Megalac-E®); SEM= standard error of the mean. p= 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; [1]Estimated by the equations of NRC (2001).

mechanisms that control food intake and the limited capacity of ruminants to oxidize fatty acids, are the main reasons for the intake inhibition (Allen *et al.*, 2005).

The results of this study contrast with the hypothesis that highly unsaturated fat sources, such as vegetable oils and calcium salts of fatty acids, reduce the nutrients digestibility. According to Jenkins (2007), the lipids in the diet could decrease fiber digestibility by forming a film that covers the food particles, avoiding the microbial adhesion, or by a direct toxic effect on cellulolytic bacteria.

Some authors observed a reduction in fiber digestibility when lipid sources were added to diets, and the magnitude of this reduction is related not only to the quantity, but mainly to the type of fatty acid present in the supplement. In this way, lipids rich in unsaturated fatty acids trend to promote higher reduction in digestibility (Fievez *et al.*, 2007).

Theoretically, the use of WS would have lower effect over nutrient digestibility, once it provides slow release of lipids on the rumen, not overcoming the hydrogenation capacity of ruminal microorganisms and avoiding the possible decrease in fiber digestibility by the negative effect of readily available unsaturated fat on rumen fibrolytic bacteria (Palmquist, 2007). The whole raw soybeans used in this study had lower concentration of EE and higher fiber content compared to what is reported in the literature (Valadares Filho *et al.*, 2010; NRC, 2001). Probably, this composition can be related to the soybean variety used, as well as to the long storing period of this oilseed. These changes in the physic-chemical composition reduced the availability of nutrients, especially the levels of TC, EE and CP for the rumen microorganisms, reducing the fermentation process and therefore the total apparent digestibility.

When analyzing the results from the present study and from the literature, it can be observed that more studies evaluating

Table IV. *Ruminal fermentation.* (Fermentação ruminal).

	Treatments				Mean	SEM	p		
	C	SO	WS	CSFA			C1	C2	C3
pH	6.66	6.75	6.77	6.84	6.76	0.03	0.031	0.674	0.196
N-NH_3 mg/100 ml	21.91	17.91	16.84	17.08	18.44	1.07	0.019	0.742	0.716
Total VFA, mol/100 mol	85.31	87.12	84.52	84.02	85.99	2.71	0.600	0.668	0.392
Acetate	59.63	60.43	59.07	60.34	59.87	1.85	0.935	0.748	0.985
Propionate	17.34	18.47	17.06	18.06	17.73	0.65	0.678	0.367	0.788
Butyrate	8.32	8.20	8.39	8.61	8.38	0.29	0.900	0.975	0.602
Total VFA, %									
Acetate	70.04	69.46	69.77	69.67	69.75	0.29	0.599	0.781	0.810
Propionate	20.28	21.09	20.27	20.44	20.52	0.30	0.608	0.453	0.390
Butyrate	9.67	9.43	9.95	9.88	9.73	0.14	0.768	0.348	0.295
C2:C3	3.49	3.32	3.46	3.47	3.41	0.06	0.869	0.665	0.989

C= control; SO= soybean oil; WS= whole raw soybeans; CSFA= calcium salts of fatty acids (Megalac-E®); SEM= standard error of the mean; p= 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.

different fat sources in specific conditions of supplementation are necessary. An interaction between fat source and level of supplementation seems to exists, affecting the response in nutrient digestibility, as well as the roughage used and cows characteristics, such as level of milk yield and stage of lactation (Shingfield and Griinari, 2007).

Fat supplementation increased ruminal pH and decreased NH_3-N concentrations, when compared to control ($p<0.05$). Although, when different fat sources were compared, there was no difference in ruminal fermentation parameters in this study ($p>0.05$; **table IV**) (Hristov *et al*., 2009).

Also, fat supplementation did not alter the VFA concentration in the rumen, either in percentage or molar proportion, when compared to the control diet ($p>0.05$; **table IV**).

Potential inhibitory effects on ruminal fermentation that are related to ammonia production, when fat sources are used in ruminant diets, have been reported (Chen and Russell, 1989). This ammonia accumulation is responsible for the inhibitory effect of unsaturated lipids on the supply of energy of carbohydrates and protein to the population of gram-positive bacteria that are obligatory amino acids fermenters (Chen and Russell, 1989). Vargas *et al*. (2002) evaluated the addition of whole soybeans and soybeans oil in diets of early lactation cows and did not verify any differences in ammonia nitrogen concentration among the fat sources evaluated. The results observed in this study indicate that the fat sources added to diets, in the levels they were used, did not influence the production of VFA (**table IV**).

When analyzing the total apparent digestibility of nutrients and ruminal fermentation data together, it can be verified that in order to evaluate the effect of feeding fat sources to dairy cows, the conditions of supplementation should be appropriately characterized, under the risk of mistaken interpretation in some situations. This way, depending on the fat source used and the

Table V. *Microbial protein synthesis.* (Sintese de proteina microbiana).

	Treatments				Mean	SEM	p		
	C	SO	WS	CSFA			C1	C2	C3
(mmol/day)									
Allantoin	259.90	250.09	266.79	282.28	266.03	17.02	0.855	0.981	0.455
Uric acid	30.63	21.49	33.94	35.53	30.40	2.69	0.956	0.373	0.053
Milk Allantoin	1.27	0.91	1.45	1.42	1.26	0.15	0.984	0.285	0.097
Total PD	287.72	285.10	294.67	316.78	296.03	17.42	0.755	0.868	0.470
Pabs	310.78	306.22	332.68	343.21	323.22	20.76	0.691	0.857	0.471
ALA: PD (%)	89.84	86.97	90.47	88.28	88.89	0.95	0.590	0.257	0.647
UV (L/day)	22.63	18.94	20.91	25.18	21.95	1.33	0.972	0.332	0.005
Nmic (g/day)	195.60	193.58	201.04	217.76	201.99	12.98	0.749	0.869	0.461
Efficiency[1]	103.56	103.10	109.45	119.91	109.00	7.36	0.637	0.899	0.377

C= control; SO= soybean oil; WS= whole raw soybeans; CSFA= calcium salts of fatty acids (Megalac-E®); SEM= standard error of the mean; p= 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; [1]TDN intake kg/g microbial crude protein.

supplementation conditions, the results of ruminal digestibility and fermentation can be different, even with similar fat sources (Allen *et al*., 2005).

Daily allantoin excretion , uric acid, milk alantoin, total purine derivatives, allantoin percentage, absorbed purines, microbial nitrogen, and microbial efficiency did not differ among the experimental diets (p>0,05; **table V**). However, animals supplemented with soybeans oil had lower total daily urine excretion, when compared to cows supplemented with CSFA.

Gozho *et al*. (2008) evaluated the effect of canola seed and linseed supplementation on the excretion of purines derivatives and total microbial nitrogen, and also did not observe effect of fat source on these variables.

Fat supplementation decreases the amount of rapidly fermentable carbohydrates, reducing the amount of total substrate for microbial protein synthesis (MPS). However, studies from the literature have reported variable responses to the use of different fat sources in dairy cows diets. In 12 studies evaluating fat supplementation and its effects over MPS, fat source did not reduce microbial efficiency and, in some cases, even increased it, five of these studies used unsaturated fat (Bach *et al*., 2005). However, such studies are still not sufficient to provide an exact conclusion about the effect of fat over MPS, since there are evidences that in diets with low amounts of roughage, the number of protozoa is decreased and consequently there is a reduction in microbial protein turnover due to defaunation, which leads to an increase in MPS efficiency.

CONCLUSION

Diets with whole raw soybeans and soybeans oil provide more efficient digestive processes, and increase milk composition of unsaturated fatty acids.

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