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Fatty Acid Composition in Blood Plasma and Follicular Liquid in Cows Supplemented with Linseed or Canola Grains

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ABSTRACT : This study was carried out to evaluate the fatty acid composition in Nellore cows supplemented with either linseed (*n*-3) or canola grains (*n*-6 and *n*-9). Fifteen Nellore cows, aged five years and bodyweight 550 kg±48 kg, were randomly distributed to the following treatments: CON (control), LIN (linseed) and CAN (canola grains). The cows were fed for 80 days. The concentrations of C18:0, C18:2 *n*-6 and C20:3 *n*-6 fatty acid were higher (p<0.10) in CON blood plasma in comparison to follicular liquid. Likewise, PUFA, *n*-6 contents, PUFA:SFA and *n*-6:*n*-3 ratios were higher (p<0.10) in blood plasma. On the other hand, C18:1 *n*-9, C22:5 *n*-3, MUFA and *n*-3 contents were lower (p<0.10) in blood plasma. C18:0, C18:2 *n*-6, C18:3 *n*-3, C22:5 *n*-3, PUFA, *n*-6, *n*-3 contents and PUFA:SFA ratio were higher (p<0.10) in LIN blood plasma than in the follicular liquid. Nevertheless, C14:0, C16:0, C16:1 *n*-7, PUFA, C16:0, C18:1 *n*-9 and MUFA contents were lower (p<0.10) in LIN blood plasma. On treatment CAN, the C18:0 and SFA contents, and *n*-6:*n*-3 ratios were higher (p<0.10) in blood plasma. However, C20:3 *n*-6, C22:5 *n*-3, PUFA and *n*-3 contents were lower (p<0.10) in blood plasma. C16:0, C18:0, PUFA, SFA contents and PUFA:SFA ratio did not differ (p>0.10) among the treatments. C14:0, C16:1 *n*-7, C18:2 *n*-6 and *n*-6 contents were higher (p<0.10) for CON and CAN than LIN. C17:1 *n*-7, C20:4 *n*-6 and C 22:0 contents were higher (p<0.10) for CAN than CON and LIN. C18:1 *n*-9, C18:3 *n*-3, MUFA and *n*-3 contents were higher (p<0.10) for LIN and CAN than CON. C20:3 *n*-6 content and *n*-6:*n*-3 ratio were higher (p<0.10) for CON than LIN and CAN. C22:5 *n*-3 content were higher (p<0.10) for CON and LIN than CAN. The concentrations of fatty acids in blood plasma and follicular liquid were not correlated for any fatty acid, independent of the treatment studied. Canola grain added to the diet of Nellore cows resulted in increased concentrations of fatty acids *n*-6 and *n*-3 in follicular liquid. (**Key Words :** Biohydrogenation, Fatty Acids, Nellore, Omega-3, Omega-6)

INTRODUCTION

According to field observations, the pregnancy rate of frozen embryos from Nellore cows is inconsistent and inferior to European Breeds such Holstein and Red Angus. In a general way, the pregnancy rate of fresh embryos is always superior to frozen embryos', what is related to cell injuries occurred during the embryo freezing process (Reichenbach et al., 2002). The cell membranes seem to be the most injured site during the embryo cooling process. According to Zeron et al. (2002), the membrane fluidity determines the concentration of the injury to be caused in the membrane during the embryo freezing process. Stubbs and Smith (1984) observed that the membrane fluidity increase is directly related to the amount of polyunsaturated fatty acids present in the plasmic membrane of the cells that will be frozen.

Furthermore, there were observed by Cavalieri et al. (2005) that worked with Holstein cows divided in two treatments: T1: Megalac® (Volac ltd., Roston, Hertfordshire, UK) and T2: linseed grains; the cows were fed with linseed or Megalac® and received embryos. No difference was observed between the treatments for the pregnancy rate in the cows. However, the authors observed that the heifers, which received embryos from cows fed with linseed, had a increased in the pregnancy rate. The authors suggested that the embryos collected and frozed from donors that were supplemented with linseed may be more resistant to freezing process than embryos from the cows supplemented with Megalac®.

Nevertheless, in ruminants some transformations may happen in the lipids of the diet, affecting the composition and the composition of the fatty acids that reach the

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duodenum, being these changes due to the process of liposis and biohydrogenation, mainly (Oliveira et al., 2004).

Less than 10% of the polyunsaturated fatty acids do not suffer biohydrogenation process in the rumen in such way that the products of the digestion that reach the small intestine are, basically, saturate fatty acids (palmitic and stearic acids), small amounts of monounsaturated fatty acids, and of small amounts of polyunsaturated fatty acids and microbial lipids (Oliveira et al., 2004). Thus, due to the process of biohydrogenation the fatty acids have their chains modified and are absorbed in the form of other fatty acids, different of those supplied on the diet (Tamminga and Doreau, 1991). According to Prado et al. (2008), fatty acid composition in *Longissimus* muscle of animals finished in feedlot and fed with fatty of less ruminal degradation (soybean and linseed) are similar to animals fed with corn. These data show that the protection of fat do not avoid the biohidrogenation in rumen and do not alter the fatty acid composition in tissues.

In monograstrics, the biohidrogenation do not occur, on the other hand this process occurs in ruminants. Fatty acid composition in tissues, in monogastric animals, is close to the feed on diet. Guillevic et al. (2009) studied the diets of the diet in monogastric animals. These authors suggest that the changes in diet of swine could be a efficient method to alter the fatty acid deposited.

On the other hand, there is little information about addiction of fatty acids in diet to alter their composition in membranes of embryos from monogastric animals. Some researchers studied only the fatty acid concentrate of oocytes, follicular liquid from oviducts of sows and cows (Khandoker et al., 1997), the lipid composition of oocytes from cows, sows and sheep (McEvoy et al., 2000). However, in these studies was not evaluated the diet influence on fatty acid composition.

The study was carried out to evaluate the fatty acids composition present in the blood stream and follicular liquid of cows supplemented with linseed or canola grains.

MATERIAL AND METHODS

Animal management

The experiment was carried out in the Experimental Farm in State University of Maringá, Paraná State, Brazil. The analyses of fatty acids composition were realized at the Laboratory of Food Analysis in State University of Maringá. There were used 15 origin pure (OP) Nellore cows with five years old, weight of 550 kg±48 kg and body score of 3 (scale from 1 to 5). The treatments were based in three experimental diets. The diet CON (control), LIN (additions of whole linseed grains) and CAN (diet with addition of whole canola grains) were used. The diets were offered during 80 days-approximately 10.5 kg/cow/d.

The diets were formulated according to the recommendations of NRC (1996) to be isoproteic and isoenergetic. There was used the controlled value (NRC, 1996) for estimating the TDN (total digestible nutrients) of the diets. The food chemical composition and the experimental diet chemical composition are presented in the Table 1. The percentages of the food in the experimental diets are presented in the Table 2. The fatty acid composition of the diets is presented in the Table 3.

Treatments and sampling

The cows were distributed in completely randomized delineation; CON treatment presented 5 repetitions (n = 5), LIN presented 5 repetitions (n = 5), and CAN treatment

Table 2. Composition of experimental diets (% DM)

Ingredients	CON[1]	LIN[2]	CAN[3]
Cottonseed hulls	47.5	69.9	69.6
Cottonseed meal	8.03	4.83	1.99
Ground corn	23.3	4.93	9.50
Corn germ	19.1	0.00	0.00
Linseed	0.00	18.4	0.00
Canola seed	0.00	0.00	16.9
Mineral salt	1.07	1.05	1.04
Limestone	1.07	1.05	1.04

[1] Control diet. [2] Diet with linseed. [3] Diet with canola.

Table 1. Chemical composition of feeds and experimental diets

Ingredients	DM[1]	CP[2]	NDF[3]	ASH[4]	EE[5]	TDN[6]
Cottonseed hulls	92.5	3.64	83.0	1.62	0.90	48.8
Cottonseed meal	93.3	34.4	51.3	4.34	1.62	63.0
Ground corn	90.7	9.51	14.7	1.22	2.56	80.0
Corn germ	84.6	14.0	26.2	3.08	6.30	77.0
Linseed	94.4	24.5	42.3	3.75	28.4	127
Canola	95.4	30.0	45.0	3.57	29.3	127
Mineral salt	97.8	-	-	-	-	-
Limestone	98.0	-	-	-	-	-
CON[7]	90.6	9.44	51.4	2.00	2.43	67.2
LIN[8]	92.9	9.10	69.1	2.08	5.96	72.1
CAN[9]	92.9	9.09	67.9	1.92	5.75	71.9

[1] Dry matter. [2] Crude protein. [3] Neutral detergent fiber. [4] Ash. [5] Ethereal extract.
[6] Total digestible nutrients. [7] Control diet. [8] Diet with linseed. [9] Diet with canola.

Table 3. Fatty acids composition in experimental diets

Fatty acids	CON[1]	LIN[2]	CAN[3]
C16:0 (hexadecanoic acid)	12.9	5.38	5.51
C18:0 (octadecanoic acid)	7.01	8.17	2.82
C18:1 *n*-9 (9-octadecenoic acid)	40.9	29.1	64.8
C18:1 *n*-7 (11-octadecenoic acid)	0.28	0.23	0.00
C18:2 *n*-6 (9,12-octadecadienoic acid)	38.1	19.3	21.3
C18:3 *n*-3 (9,12,15-octadecatrienoic acid)	0.85	37.8	5.53
Polyunsaturated fatty acids	38.9	57.1	26.8
Monounsaturated fatty acids	41.2	29.4	64.8
Saturated fatty acids	19.9	13.6	8.33
n-6 (Omega-6)	38.1	19.3	21.3
n-3 (Omega-3)	0.85	37.8	5.53
PUFA:MUFA	1.95	5.03	3.23
n-6:*n*-3	44.9	0.52	3.86

[1] Control diet. [2] Diet with linseed. [3] Diet with canola.

presented 5 repetitions (n = 5).

After 70 days of the experiment, the cows were submitted to oestrus synchronization with an auricular implant containing 5.0 mg of Norgestomet (Crestar®, Lab. Intervet Ltda) and intramuscularly administration of 2.0 mg of estradiol benzoate (Estrogin®, Lab. Farmavet Ltda), being considered the first day of the hormonal protocol or day 0 (D0). In D9, the auriculars implants were removed and there was intramuscularly given, in the morning, 2.0 ml of luteolytic hormone, prostaglandin F2α (PGF2α) (Ciosin®, Lab. Coopers Ltda). In the morning of D11, blood was sampled and follicular liquid aspirated.

Samples of blood were taken through jugular puncture in heparinized tubes, being afterwards submitted to centrifugation at 3,500 rpm during 10 minutes. The blood plasma was separated, added in *eppendorf* flasks in duplicate of 2 ml each and stored at -20°C until further analyses. The aspiration of the follicular liquid was performed through an ultra-sound device (ALOKA type SSD500) with convex sectorial probe of 5 MHz adapted to a needle system (18 G) and coupled to the vacuum system (pump) with follicular aspiration corresponding to 13-15 ml of water per minute. The follicular liquid was aspirated in 50 ml corning tube and after transferred to *eppendorf*.

Extraction of the fatty acids

The transesterification of the triacylglycerols for the obtention of metiyl esters of fatty acids was realized according to the methodology described by Folch (1957).

There was used Shimadzu A-14 chromatograph equipped with a detector of a flame ionization and capillary column of silica merged (100 m of length, 0.25 mm of internal diameter and 0.2 μm of CP-Sil88, ChromPack). The flows of the gases were the following: 1.2 ml/min for the straining gas H_2; 30 ml/min for the auxiliary gas N_2; and 300 ml/min for the gases of the flame H_2 and synthetic air, respectively. The temperatures of the injector and detector were, respectively, 220 and 230°C. The column temperature was 60°C during 4 minutes, being afterwards increased to 140°C, at a rate of 40°C/min, in which was maintained for ten minutes. Afterwards, the temperature was raised to 225°C, at a rate of 5°C/min, maintained for 12 minutes. The areas of peak were determined by the normalization method using a processor CG-300. The peaks were identified by comparison of the times of retention of the methyl esters and fatty acids patterns (Sigma).

Statistical analysis

The analysis statistical were analyzed using the software SAS.

The statistical model employed was the following:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

where:

Y_{ij} = Observation regarding the animal "j", submitted to the treatment i (i = 1, 2, and 3);

μ = General constant;

T_i = Effect of the treatment i (i = 1, 2, and 3);

e_{ijk} = Random error associated to each observation.

The Tukey test was used for the comparison between the means at 10%.

RESULTS AND DISCUSSION

Fatty acids composition of blood plasma and follicular liquid in control treatment

C14:0, C16:0, C16:1 *n*-7, C17:1 *n*-7, C18:3 *n*-3, C20:4 *n*-6, C22:0, saturated fatty acids (SFA) percentages did not have difference ($p>0.10$) between blood plasma (PLA) and follicular liquid (FLI) in control treatment (CON) (Table 4).

The percentage of C18:0, C18:2 *n*-6, C20:3 *n*-6, polyunsaturated fatty acids (PUFA), *n*-6 and PUFA:SFA were higher ($p<0.10$) in PLA than in FLI.

Table 4. Fatty acids composition of blood plasma and follicular liquid in control treatment (CON)

Fatty acids	PLA[1]	FLI[2]	p<f
C14:0 (tetradecanoic acid)	1.55±0.23	1.43±0.21	ns
C16:0 (hexadecanoic acid)	19.9±2.83	19.4±2.78	ns
C16:1 *n*-7 (9-hexadecenoic acid)	0.34±0.05	0.27±0.04	ns
C18:0 (octadecanoic acid)	22.2±1.05	18.3±0.86	0.10
C18:1 *n*-9 (9-octadecenoic acid)	14.5±1.30	27.4±2.12	0.10
C18:2 *n*-6 (9,12-octadecadienoic acid)	16.1±1.17	4.71±0.29	0.10
C18:3 *n*-3 (9,12,15-octadecatrienoic acid)	0.00±0.00	0.00±0.00	ns
C20:3 *n*-6 (8,11,14-eicosatrienoic acid)	2.01±0.53	0.22±0.06	0.10
C20:4 *n*-6 (5,8,11,14-eicosatetraenoic acid)	0.27±0.20	0.31±0.07	ns
C22:5 *n*-3 (7,10,13,16,19-docosapentaenoic acid)	0.31±0.04	1.58±0.12	0.10
Polyunsaturated fatty acids	18.7±3.69	6.82±0.34	0.10
Monounsaturated fatty acids	14.8±1.39	27.7±2.51	0.10
Saturated fatty acids	43.6±4.08	39.2±3.67	ns
n-6 (Omega-6)	18.4±0.59	5.24±0.21	0.10
n-3 (Omega-3)	0.31±0.13	1.58±0.31	0.10
PUFA:SFA	0.43±0.13	0.17±0.05	0.10
n-6:*n*-3	59.4±2.80	3.32±0.15	0.10

[1] Blood plasma. [2] Follicular liquid.

There was an increase in percentage of C18:0 in blood plasma and follicular liquid in relation to the percentage of the acid (7.01%) of the diet. Such result may be associated to the biohydrogenation process, in which, during this process, the glycerol is fermented to propionic acid and the unsaturated fatty acids hydrogenated to saturated fatty acids.

The higher percentage of C18:2 *n*-6 in diet could be the reason of the highest concentration of C18:0, since the hydrogenation of C18:2 *n*-6 results in the formation of C18:0 as well as the different isomers of C18:1 (Tamminga and Doreau, 1991). The main source of C18:2 *n*-6 used in this work was the corn, which was grounded, therefore favouring the exposition of this fatty acid to the process of biohydrogenation in the rumen.

Other aspect that should be observed is the lower concentrations of PUFA and MUFA in blood plasma and in follicular liquid in relation to the percentages of the diets. The contrary can be observed in saturated fatty acids (SFA), which presented lower percentages in diet. The reduction in PUFA and MUFA percentages, and consequently, the increase in SFA percentage indicate the saturation due to the ruminal process.

C20:3 *n*-6 and C20:4 *n*-6 were not offered in diet. But theses fatty acids were found in blood plasma and follicular liquid. This can be due the metabolism of C18:2 *n*-6. According to Souza and Visentainer (2006), C18:2 *n*-6 may suffer the action of the enzyme D6-dessaturase, resulting C18:3 *n*-6. More, according to the same authors, C20:3 is formed by the action of the enzyme alongase. Thus, C20:3 *n*-6 may originate the prostaglandins of the series 1, the thromboxane A1 and part of it may form the C20:4 *n*-6 by the action of the enzyme D5-dessaturase. These enzymes are important to the reproduction, because they help the environment of the pregnancy (Souza and Visentainer, 2006).

Fatty acids composition of blood plasma and follicular liquid in linseed treatment

C17:1 *n*-7, C20:3 *n*-6, C20:4 *n*-6, C22:0, SFA percentages and *n*-6:*n*-3 ratio did not differ (p>0.10) between PLA and FLI in linseed treatment (Table 5).

The percentage of C18:0, C18:2 *n*-6, C18:3 *n*-3, C22:5 *n*-3, PUFA, *n*-6, *n*-3 and *n*-6:*n*-3 ratio were higher (p<0.10) in PLA than in FLI.

In blood plasma of cows in LIN treatment, the percentage of C14:0, C16:0, C16:1 *n*-7, C18:1 *n*-9 and MUFA were lower (p<0.10) than in follicular liquid.

Similarly to the occurrence in blood plasma and follicular liquid in CON treatment, C16:0 and C18:0 were higher in blood plasma and follicular liquid than the diet offered to LIN treatment.

These results may be associated not only with the metabolism of C18:2 *n*-6, but also with the metabolism of C18:3 *n*-3. Normally, the product of the hydrogenation of C18:3 *n*-3 is C18:0, while the hydrogenation of C18:2 *n*-6 produces only C18:2 *n*-6 as well as in C18:1 (Tamminga and Doreau, 1991).

According to Oliveira et al. (2004), to reduce the biohydrogenation and increase the amount of PUFA in intestine by the diet is necessary to offer diets rich in PUFA, but also elevate the ruminal pH. Palmquist and Jenkins (1980) cited that cellulolytic bacteria are the microorganisms responsible by the biohydrogenation since they are the most affected by the supplementation with fat and by the decrease of pH. Harfoot and Hazlewood (1997) observed that the replacement of the fibre in diet by

Table 5. Fatty acids composition of blood plasma and follicular liquid in linseed treatment (LIN)

Fatty acids	PLA[1]	FLI[2]	p<f
C14:0 (tetradecanoic acid)	0.70±0.17	4.12±0.50	0.10
C16:0 (hexadecanoic acid)	20.7±2.00	30.8±2.27	0.10
C16:1 *n*-7 (9-hexadecenoic acid)	0.00±0.00	1.70±0.12	0.10
C18:0 (octadecanoic acid)	32.0±2.66	19.9±1.66	0.10
C18:1 *n*-9 (9-octadecenoic acid)	24.6±1.47	31.5±1.89	0.10
C18:2 *n*-6 (9,12-octadecadienoic acid)	11.4±0.84	5.86±0.49	0.10
C18:3 *n*-3 (9,12,15-octadecatrienoic acid)	2.67±0.09	0.42±0.03	0.10
C20:3 *n*-6 (8,11,14-eicosatrienoic acid)	0.17±0.04	0.00±0.00	ns
C20:4 *n*-6 (5,8,11,14-eicosatetraenoic acid)	0.78±0.06	0.69±0.05	ns
C22:5 *n*-3 (7,10,13,16,19-docosapentaenoic acid)	0.24±0.04	0.00±0.00	0.10
Polyunsaturated fatty acids	15.3±1.74	6.97±0.69	0.10
Monounsaturated fatty acids	24.6±1.96	33.1±1.97	0.10
Saturated fatty acids	53.5±3.06	54.8±3.94	ns
n-6 (Omega-6)	12.5±1.03	6.55±0.56	0.10
n-3 (Omega-3)	2.91±0.36	0.42±0.05	0.10
PUFA:SFA	0.36±0.08	0.13±0.03	0.10
n-6:*n*-3	4.28±0.41	5.64±0.62	ns

[1] Blood plasma. [2] Follicular liquid.

carbohydrate of rapid ruminal degradation resulted in reduction of liposis and biohydrogenation rates, suggesting action of cellulolytic microorganisms upon the biohydrogenation process.

Fatty acids composition of blood plasma and follicular liquid in canola treatment

C14:0, C16:0, C16:1 *n*-7, C17:1 *n*-7, C18:1 *n*-9, C18:2 *n*-6, C18:3 *n*-3, C20:4 *n*-6, C22:0, MUFA, *n*-6 percentages and PUFA:SFA ratio did not differ (p>0.10) between PLA and FLI in canola (CAN) treatment (Table 6).

In CAN treatment, the percentage of C18:2 *n*-6 in blood plasma and follicular liquid were close to the percentage offered in the diet.

The percentage of C18:0, SFA, and *n*-6:*n*-3 ratio were higher (p<0.10) in PLA than FLI. In FLI the C20:3 *n*-6, C22:5 *n*-3, PUFA and *n*-3 percentages were higher (p<0.10) than in PLA.

The oleaginous seeds are considerably used due to the high concentrations of lipids and interesting characters regarding the oil release rate (Prado et al., 2008). That oil is released as the animal consumes through chewing, sending

Table 6. Fatty acids composition of blood plasma and follicular liquid in canola treatment (CAN)

Fatty acids	PLA[1]	FLI[2]	p<f
C14:0 (tetradecanoic acid)	1.89±0.15	1.50±0.13	ns
C16:0 (hexadecanoic acid)	19.9±1.13	19.2±1.17	ns
C16:1 *n*-7 (9-hexadecenoic acid)	0.61±0.10	0.93±0.12	ns
C17:1 *n*-7 (10-heptadecenoic acid)	0.62±0.01	0.73±0.09	ns
C18:0 (octadecanoic acid)	27.0±0.26	22.9±0.24	0.10
C18:1 *n*-9 (9-octadecenoic acid)	26.8±0.20	23.7±0.23	ns
C18:2 *n*-6 (9,12-octadecadienoic acid)	16.5±0.11	15.1±0.13	ns
C18:3 *n*-3 (9,12,15-octadecatrienoic acid)	2.04±0.18	1.82±0.14	ns
C20:3 *n*-6 (8,11,14-eicosatrienoic acid)	0.98±0.04	1.85±0.09	0.10
C20:4 *n*-6 (5,8,11,14-eicosatetraenoic acid)	1.26±0.10	1.33±0.15	ns
C22:0 (docosanoic acid)	0.41±0.07	0.32±0.09	ns
C22:5 *n*-3 (7,10,13,16,19-docosapentaenoic acid)	0.00±0.00	3.44±0.21	0.10
Polyunsaturated fatty acids	20.8±0.98	26.1±1.01	0.10
Monounsaturated fatty acids	27.5±1.02	25.5±1.00	ns
Saturated fatty acids	49.3±1.20	43.8±1.14	0.10
n-6 (Omega-6)	18.7±0.78	18.3±0.76	ns
n-3 (Omega-3)	2.04±0.12	5.26±0.34	0.10
PUFA:SFA	0.42±0.04	0.60±0.07	ns
n-6:*n*-3	9.17±0.35	3.48±0.12	0.10

[1] Blood plasma. [2] Follicular liquid.

little fractions to the ruminal environment (Coppock and Wilks, 1991). Thus, even the seed or grain being a protected way to supply unsaturated fatty acids, its protection cannot be totally guaranteed.

In a general way, independently of the treatments, the blood plasma and follicular liquid not have correlated percentages for some fatty acids.

Yao et al. (1980) observed, in study with sows, differences in the concentration of fatty acids in function to the size of the follicle (1-2 mm; 3-5 mm, and 6-12 mm) for C20:4 *n*-6, being that concentration increased as result of the follicular maturation. They related also that the concentrations of C18:0 and C18:1 were higher in the blood serum in relation to the follicular liquid;-similar results for the concentrations of C 18:0 to those obtained in this study.

Fatty acids of blood plasma in relation of the addition linseed and canola

C16:0, C18:0, PUFA, SFA percentages and PUFA:SFA ratio did not differ (p>0.10) among CON, LIN and CAN treatments (Table 7).

The percentage of C14:0, C16:1 *n*-7, C18: 2 *n*-6 and n-6 were higher (p<0.10) in CON and CAN than in LIN treatment.

C18:1 *n*-9, C18:3 *n*-3, MUFA and *n*-3 contents were higher (p<0.10) in LIN and CAN than in CON treatment.

C20:3 *n*-6 percentage and *n*-6:*n*-3 ratio were higher (p<0.10) in CON than in LIN and CAN treatments. The highest ratio of *n*-6:*n*-3 for CON treatment is due to the fact of this treatment presenting lower (p<0.10) percentage of fatty acids *n*-3 in relation to LIN and CAN.

The percentage of C17:1 *n*-7, C20:4 *n*-6 and C22:0 were higher (p<0.10) in CAN than in CON and LIN treatments.

C22:5 *n*-3 percentage was higher (p<0.10) in CON and LIN than in CAN treatment.

Petit (2003), added grains of linseed or sunflower's seeds or only sunflower's seeds treated with formaldehyde in Holstein cows, observed that the concentrations of fatty acids were affected by the inclusion of seeds. The concentrations in the blood plasma increased for C14:0, C16:0, C18:1 *n*-9, C18:3 *n*-3, and C20:5 *n*-3 due to additions of grains of linseed to sunflower's seeds. Regarding the treatment with formaldehyde, the authors observed higher concentrations for C18:1 *n*-9 and C18:2 *n*-6. The ratio of the *n*-6:*n*-3, was lower in blood plasma of cows that have consumed linseed.

In this study, *n*-6:*n*-3 ratio was higher in three treatments (CON, LIN, and CAN) when compared to values observed by Petit (2003) with regard to the linseed's seed whether treated or not with formaldehyde (2.7) in both cases. However, *n*-6:*n*-3 ratio was similar to the values obtained with the addition of sunflower's seed whether treated or not with formaldehyde (11.9 and 11.0).

Fatty acids of follicular liquid in relation of the addition linseed and canola

C18:0 percentage did not differ (p>0.10) among CON, LIN and CAN treatments (Table 8).

The percentage of C14:0, C16:0, C16:1 *n*-7, C18:1 *n*-9, MUFA, SFA and *n*-6:*n*-3 ratio were higher (p<0.10) in LIN than in CON and CAN treatments.

n-6:*n*-3 ratio has direct implication to the embryo's

Table 7. Fatty acids composition of blood plasma in relation of the addition linseed and canola

Fatty acids	CON[1]	LIN[2]	CAN[3]	p<f
C14:0 (tetradecanoic acid)	1.55±0.36[a]	0.70±0.16[b]	1.89±0.44[a]	0.10
C16:0 (hexadecanoic acid)	19.6±2.70	20.7±2.85	19.9±2.74	ns
C16:1 n-7 (9-hexadecenoic acid)	0.34±0.05[a]	0.00±0.00[b]	0.31±0.05[a]	0.10
C17:1 n-7 (10-heptadecenoic acid)	0.00±0.00[b]	0.00±0.00[b]	0.32±0.05[a]	0.10
C18:0 (octadecanoic acid)	22.2±1.59	32.1±2.29	27.0±1.92	ns
C18:1 n-9 (9-octadecenoic acid)	14.5±1.43[b]	24.6±2.43[a]	26.8±2.65[a]	0.10
C18:2 n-6 (9,12-octadecadienoic acid)	16.1±1.25[a]	11.4±1.30[b]	16.5±1.33[a]	0.10
C18:3 n-3 (9,12,15-octadecatrienoic acid)	0.00±0.00[b]	2.67±0.41[a]	2.04±0.31[a]	0.10
C20:3 n-6 (8,11,14-eicosatrienoic acid)	2.01±0.80[a]	0.27±0.11[c]	0.98±0.39[b]	0.10
C20:4 n-6 (5,8,11,14-eicosatetraenoic acid)	0.27±0.10[c]	0.78±0.29[b]	1.26±0.47[a]	0.10
C22:0 (docosanoic acid)	0.00±0.00[b]	0.00±0.00[b]	0.51±0.08[a]	0.10
C22:5 n-3 (7,10,13,16,19-docosapentaenoic acid)	0.31±0.09[a]	0.24±0.02[a]	0.00±0.00[b]	0.10
Polyunsaturated fatty acids	18.7±3.48	15.4±2.87	20.8±3.88	ns
Monounsaturated fatty acids	14.8±1.44[b]	24.6±2.41[a]	27.5±2.69[a]	0.10
Saturated fatty acids	43.6±4.15	53.5±5.10	49.3±4.70	ns
n-6 (Omega-6)	18.4±3.51[a]	12.5±2.38[b]	18.7±3.56[a]	0.10
n-3 (Omega-3)	0.31±0.08[b]	2.91±0.68[a]	2.04±0.48[a]	0.10
PUFA:SFA	0.43±0.11	0.36±0.09	0.42±0.11	ns
n-6:*n*-3	59.4±3.24[a]	4.28±0.23[b]	9.17±0.50[b]	0.10

[1] Control diet. [2] Diet with linseed. [3] Diet with canola.

Table 8. Fatty acids composition of follicular liquid in relation of the addition of linseed and canola

Fatty acids	CON[1]	LIN[2]	CAN[3]	p<f
C14:0 (tetradecanoic acid)	1.43±0.30[b]	4.12±0.87[a]	1.50±0.32[b]	0.10
C16:0 (hexadecanoic acid)	19.4±1.45[b]	30.8±2.30[a]	19.2±1.43[b]	0.10
C16:1 *n*-7 (9-hexadecenoic acid)	0.27±0.06[c]	1.70±0.37[a]	0.93±0.20[b]	0.10
C17:1 *n*-7 (10-heptadecenoic acid)	0.00±0.00[b]	0.00±0.00[b]	0.93±0.14[a]	0.10
C18:0 (octadecanoic acid)	18.3±0.97	19.9±1.05	22.9±1.21	ns
C18:1 *n*-9 (9-octadecenoic acid)	27.4±1.33[b]	31.5±1.53[a]	23.7±1.15[b]	0.10
C18:2 *n*-6 (9,12-octadecadienoic acid)	4.71±0.81[b]	5.86±1.00[b]	15.1±2.58[a]	0.10
C18:3 *n*-3 (9,12,15-octadecatrienoic acid)	0.00±0.00[c]	0.42±0.11[b]	1.82±0.49[a]	0.10
C20:3 *n*-6 (8,11,14-eicosatrienoic acid)	0.22±0.04[b]	0.00±0.00[c]	1.85±0.36[a]	0.10
C20:4 *n*-6 (5,8,11,14-eicosatetraenoic acid)	0.31±0.06[c]	0.69±0.14[b]	1.33±0.27[a]	0.10
C22:0 (docosanoic acid)	0.00±0.00[b]	0.00±0.00[b]	0.22±0.03[a]	0.10
C22:5 *n*-3 (7, 10, 13,16,19-docosapentaenoic) acid)	1.58±0.68[b]	0.00±0.00[c]	3.44±1.58[a]	0.10
Polyunsaturated fatty acids	6.82±0.57[b]	6.97±0.58[b]	26.1±2.21[a]	0.10
Monounsaturated fatty acids	27.7±1.49[b]	33.2±1.78[a]	25.5±1.37[b]	0.10
Saturated fatty acids	39.2±1.80[b]	54.8±2.52[a]	43.8±2.01[b]	0.10
n-6 (Omega-6)	5.24±0.78[b]	6.55±0.97[b]	18.3±2.73[a]	0.10
n-3 (Omega-3)	1.58±0.29[b]	0.42±0.13[c]	5.26±1.66[a]	0.10
PUFA:SFA	0.17±0.01[b]	0.13±0.01[b]	0.60±0.05[a]	0.10
n-6:*n*-3	3.32±0.60[b]	5.64±1.02[a]	3.48±0.63[b]	0.10

[1] Control diet. [2] Diet with linseed. [3] Diet with canola seed.

freezing process. According to Zeron et al. (2002), the fluidity of the membrane determines the concentration of the lesion during the freezing process. More, the fluidity of the membrane may be associated to a higher performance of intracellular water exchange by the cryoprotector, therefore, favouring the embryo protection, consequently, would increase the gestation rates of frozen embryos.

Stubbs and Smith (1984) observed that the membrane's fluidity increase is directly related to the amount of polyunsaturated fatty acids present in the blood plasma membrane of cells that will be frozen.

C17:1 *n*-7, C18:2 *n*-6, C18:3 *n*-3, C20:3 *n*-6, C20:4 *n*-6, C22:0, C22:5 *n*-3, PUFA, *n*-6, *n*-3 percentages and PUFA:SFA ratio were higher ($p<0.10$) in CAN than in CON and LIN treatments.

In the follicular liquid of cows that received the diet containing grains of linseed, there was an increase in concentration of SFA and consequently, lower ratio between the PUFA and SFA. Thus, the chewing process itself might have caused the partial or total breakage of the grains, favouring the process of biohydrogenation.

N-6 fatty acids had lower percentage in follicular liquid. This result can be contradictory when offering a source of high concentrations of these acids in the diet.

Petit (2002) observed that the whole grains of linseeds hindered the digestibility of EE (ether extract) and the CP (crude protein), compared with supply of Megalac® or soybean. For both sources, it may have occurred greater access of the microorganisms and enzymes to the fat in relation to the whole linseed grains.

According to this author, in the grains of linseed, the fat would be associated to a protein-fibrous matrix present in the grain. Further, when the fatty acids are associated to cellular structures of the aliments, they are not fully available, hindering the formation of micellae for the digestion (Bauchart, 1993).

Zeron et al. (2001) observed differences in concentrations of fatty acids in oocytes, rough cells and follicular liquid of Holstein cows during the winter and summer. During the summer, there was observed a high concentration of saturate fatty acids in the oocytes (C16:0), rough cells and follicular liquid (C16:0 and C18:0). On the other hand, the concentration of PUFA (C18:2 *n*-6 and 22:6 *n*-3) was higher in the oocytes and rough cells during winter. Higher percentage of C20:4 *n*-6 was obtained in the summer for rough cells and follicular liquid. However, lower concentration of fatty acids was observed in oocyte in the same season. These authors highlighted the importance of C20:4 *n*-6 because it is a prostaglandin precursor.

Zeron et al. (2001) did not observe the presence of C22:6 *n*-3. Similarly, the authors did not observe the presence of C20:5 *n*-3. Nevertheless, this fatty acid was observed in the work despite its low concentration.

IMPLICATIONS

The concentrations of fatty acids in blood plasma and follicular liquid are not correlated for some fatty acids, independent of the treatment studied. The grains of linseed - rich source of fatty acids *n*-3 - did not alter the concentrations in follicular liquid. However, grains of canola added to the diet of Nellore cows resulted in increase

of concentrations of fatty acids *n*-6 and *n*-3 in follicular liquid.

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