



Pelleting in Associated with Sodium Monensin Increases the Conjugated Linoleic Acids Concentration in the Milk of Dairy Cows Fed Canola Seeds

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ABSTRACT: To evaluate the effects of the pelleting and the addition of sodium monensin on production, the chemical and lipid composition of milk and butter physical characteristics, 4 Holstein dairy cows (135 days of lactation) with an average milk production of 14.7 kg/d, were supplemented with a concentrate containing ground canola seeds. The cows were assigned to a 4×4 Latin square design with a 2×2 factorial arrangement of treatments: i) ground maize, soybean meal, mineral and vitamin supplements, and ground canola seeds (CG); ii) CG concentrate with 31.5 mg of monensin added per kg of dry matter (DM); iii) CG pelleted concentrate; iv) CG concentrate with monensin addition pelleted. There was no difference in milk production and composition. The addition of monensin increased milk concentration of polyunsaturated fatty acids (PUFA), the PUFA/saturated fatty acids (SFA) ratio, and omega 6. The pelleting increased the concentration of monounsaturated fatty acids, the PUFA/SFA ratio, and the omega 6/omega 3 ratio, but decreased the concentration of SFA. The association between pelleting and the addition of monensin increased the concentration of conjugated linoleic acids by 46.9%. The physical characteristics of butter were not affected by the evaluated diets. We concluded that the concentrate with 31.5 mg of monensin added per kg DM basis combined with the pelleting improves the lipid composition of milk from Holstein cows that are on pasture and supplemented with ground canola seeds, without changing the production, milk composition, and spreadability of butter. (**Key Words:** Animal Production, Ionophore, Heat Treatment, Polyunsaturated Fatty Acid)

INTRODUCTION

Fatty acids (FA) of the omega 3 and omega 6 families as well as conjugated linoleic acids (CLA) and its isomers have been shown to have an important function in preventing human diseases (Cichosz and Czczot, 2012). Ruminant's milk fat is the richest CLA natural source, and in bovine milk the isomer C18:2 *cis-9 trans-11* represents an average 90% of the known isomers and it can be influenced by many sources of variations (Parodi, 2009).

The addition of oilseeds or their oils in the diet of dairy cows have been effective in improving milk fatty acid profile because they decrease saturated fatty acids (SFA)

and increase polyunsaturated fatty acids (PUFA) levels in the form of CLA, omega 3, and omega 6 FA (Mohammed et al., 2011). Another factor that can potentiate this increase is keeping animals on pasture (Schroeder et al., 2004); because α -linolenic and linoleic FA are abundant in grasses. These FA are CLA precursors during the ruminal biohydrogenation process and in the mammary gland.

Canola is an oilseed that, despite the high oil content (55%), is considered a protein source for ruminants (20% crude protein [CP]) and is rich in PUFA. Based on the total FA, the oil is composed of around 60% oleic acid (omega 9), 20% linoleic acid (omega 6), and 10% linolenic acid (omega 3; Canola Council of Canada, 2015). However, canola seed has low digestibility, unless the hull is broken and the core exposed. In this case, PUFA are exposed for ruminal biohydrogenation. Thus, if ruminal biohydrogenation can be controlled, it will be possible to

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increase the transfer of these PUFA for milk and milk derivatives.

The processing of concentrated feeds via thermal treatments, as the pelleting, is an alternative method to protect canola PUFA from ruminal biohydrogenation, and to improve nutrient digestibility of the diet (Neves et al., 2007; Neves et al., 2009; dos Santos et al., 2011). Another alternative is the use of ionophores that, in addition to improving energy metabolism, reducing the ruminal degradation of dietary protein, increasing the digestibility and milk production, also reduce SFA and increase CLA content in the milk of dairy cows (Duffield et al., 2008; da Silva-Kazama et al., 2011). Despite the recent questionings from the World Health Organization about the addition of antibiotics in animal feed and the preventive prohibition on its use by the European Union in 2006 (Regulation [EC] No 1831/2003), monensin is a feed additive permitted by US Food and Drug Administration since 2005 (FDA, 2005, Freedom of Information Summary - NADA 095-735) and by Food and Agriculture Organization of the United Nations (FAO). Data collected by FAO show that the monensin, when used in the appropriate doses, doesn't leave residues in ruminant milk or remains below to maximum residue limits, not being in that way a risk to human health (Bizec and Sanders, 2009). In Brazil, Australia, Canada, New Zealand and others countries monensin addition in animal feed is permitted. Thus, our hypothesis is that the pelleting and the addition of monensin can increase milk production and improve milk lipid composition; moreover, these effects can be potentiated through the association of the two treatments. Therefore, we aimed to evaluate the effects of the pelleting and the addition of monensin on intake, apparent nutrient digestibility, milk production, milk chemical and lipid composition, and butter texture in Holstein cows on pasture and supplemented with concentrate containing ground canola seeds.

MATERIALS AND METHODS

Animals and experimental diets

This study was conducted at the experimental farm of the Universidade Estadual de Maringá, southern Brazil. The Animal Care and Use Committee of the Universidade Estadual de Maringá approved all animal procedures. Four primiparous Holstein cows (134.8±64 lactation days), with an average weight of 449.8±5.3 kg in the beginning of the experiment and an average milk production of 14.7±1.4 kg/d were distributed in a Latin square design with four 21-d periods, which was comprised of 16 days for acclimation and 5 days for collections. The animals were kept on rotational grazing with free access to water. Twice daily (6:00 h and 15:00 h), cows were taken to be milked, after which they were housed in a tie stall type system, for

around 3 h, in order to provide the concentrate. The concentrate treatments were: i) ground corn, soybean meal, mineral and vitamin supplements, and ground canola seeds (CG); ii) concentrate CG with the addition of 31.5 mg of monensin/kg dry matter (DM) of concentrate (CGM); iii) concentrate CG pelleted (CGP); iv) concentrate CG with monensin addition pelleted (CMP) (Table 1). The canola and corn grains were ground through a 5 mm screen using a Nogueira DPM-2 chopper (Irmãos Nogueira S.A. Máquinas Agrícolas e Motores, Itapira, São Paulo, Brazil). Monensin was provided with the mineral vitamin supplement, which contained 480 mg of monensin per kg of product. The amount of monensin added was based in the monensin concentration in mineral vitamin supplement and in the amount normally used in practice by milk farmers. The same mineral vitamin supplement, but without monensin, was used for the treatment without ionophores. The 40HP pelletizer was utilized for the pelleting (Chavantes, Chavantes, SP, Brazil) located at the Iguatemi Experimental Farm, UEM, the ration's temperature checked at the end of process was around 95°C to 98°C. The rations were balanced to provide isonitrogenous and isocaloric diets (Table 1) to meet Holstein dairy cows' requirements according to (NRC, 2001).

The pasture area consisted of four 0.4 ha paddocks with grasses of the *Cynodon* genus (Coast-cross, Tifton 85 and African star predominance). These grasses contained an average composition of 270.1±15 g/kg DM, 163.8±3 g/kg CP, 22.1±3 g/kg ether extract (EE), 664.8±46 g/kg neutral detergent fiber (NDF), 315±26 g/kg acid detergent fiber (ADF), and 28.4±1.2 g/kg lignin. The average precipitation was 175.4 mm during the trial, with an average temperature of 26°C (Maringá Main Climatological Station [ECPM] located at UEM).

The handling of the animals in the paddocks was determined by pasture height, such that the animals were placed in the paddocks when the grasses achieved an average height of 40 cm and they were removed from the paddocks when the grasses had an average height of 20 cm. The sward height was measured at 10 different points in each paddock; pre-grazing heights were based on the length from soil level until the last leaf curvature, and the heights for post-grazing were measured on the length from soil level. In average the permanence of animals in each paddock was 2 d.

Experimental procedures

The available forage mass was, on average, 1.7 ton DM/ha, and was estimated by using samples that were collected when the animals entered the paddocks, using a square (1.0 m²) in four points in each paddock from 5 cm of soil. For proximate composition, samples of the grazed stratum above the post grazing residue were manually

Table 1. Ingredients of feed concentrates and chemical composition of total diets

Items	Non-pelleted		Pelleted ¹	
	CG	CGM	CGP	CMP
Ingredients				
Ground maize (g/kg of DM)	571.4	571.4	571.4	571.4
Soybean meal (g/kg of DM)	54.3	54.3	54.3	54.3
Ground canola seeds (g/kg of DM)	285.7	285.7	285.7	285.7
Limestone (g/kg of DM)	7.1	7.1	7.1	7.1
Calcium phosphate (g/kg of DM)	11.4	11.4	11.4	11.4
Salt (g/kg of DM)	0.6	0.6	0.6	0.6
Mineral and vitaminic supplement (g/kg of DM) ²	65.7	65.7	65.7	65.7
Sodium bicarbonate (g/kg of DM)	3.7	3.7	3.7	3.7
Sodium monensin (mg/kg)	-	31.54	-	31.54
Chemical composition of total diet				
Dry matter (g/kg)	557.9	559.1	557.5	558.2
Crude protein	155.8	154.9	157.4	155.8
Ether extract	72.9	74.6	67.4	67.4
Neutral detergent fiber	431.8	434.4	432.2	434.3
Acid detergent fiber	207.4	205.4	206.3	204.5
Non-fibrous carbohydrate	273.6	266.2	274.1	276.3

¹ CG, feed concentrate with ground canola seeds; CGM, feed concentrate CG with sodium monensin addition; CGP, feed concentrate CG pelleted; CMP, feed concentrate CG with sodium monensin addition and pelleted.

² Ca, 156 g/kg; P, 51 g/kg; S, 20 g/kg; Na, 93 g/kg; K, 28 g/kg; Mg, 33 g/kg; Fe, 2,000 mg/kg; Cu, 400 mg/kg; Co, 30 mg/kg; Cr, 10 mg/kg; I, 40 mg/kg; Se, 15 mg/kg; Zn, 1,700 mg/kg; F, maximum 510 mg/kg; Mn, 1,350 mg/kg; Vit. A, 135,000 UI/kg; Vit. D, 68,000 UI/kg; Vit. E, 450 mg/kg.

collected from several points in each paddock. Samples of pasture were pooled based on period and paddock to obtain one sample/period.

To determine the dry matter intake (DMI) and nutrient intake of the concentrate, the amount of concentrate offered was recorded daily such as orts. Concentrate intake was adjusted daily until the beginning of the collection week, and it was limited based on the estimated intake of 3.0% body weight, while a roughage:concentrate ratio of 60:40 was offered. During the collection periods, concentrate and orts were sampled daily. At the end of each experimental period, a sample pool was performed, thereby resulting in a unique sample per animal per period. Roughage intake was estimated using the indigestible dry matter (iDM) as an internal marker, and was estimated in the samples of concentrate, orts and feces. To estimate the daily fecal excretion, we utilized chromium oxide (Cr₂O₃) as an external marker, which was provided orally in cellulose bags from the 5th to 15th day of each experimental period. Chromium oxide was given twice daily (5 g each), thus totaling 10 g of external marker per animal per day (Detmann et al., 2001). For the apparent digestibility assay, fecal samples were collected directly from the rectum from the 10th to 15th day of the experimental period, twice daily (8:00 h and 16:00 h), and a composed sample was proportionally performed on a dry weight basis per animal/period for further analyses.

Milk production was recorded daily and corrected for 3.5% fat content of milk. Milk was sampled on the 17th and

18th day of each experimental period, and a composite sample was proportionally performed in relation to the milk production in the morning and in the afternoon. The sample was then stored in a plastic bottle with the preservative 2-bromo 2-nitropropano 1-3-diol (bromopol), for the analyses of dry extract, CP, fat, somatic cell count (SCC), and urea. A portion of the composite sample was stored in a plastic bottle and frozen at -20°C for lipid composition analysis. Also, measurements of milk acidity and density were performed.

For the butter fabrication, 10 liters of milk from each animal were collected in a ratio that was proportional to the milk production in the morning and in the afternoon. The milk was kept at 4°C for 2 days for the precipitation of fat, which was removed and stored in plastic bottles at -10°C over one day. After this period, the fat was processed in a mixer to obtain the butter, which was then stored in plastic bottles under refrigerated conditions for further analyses.

Laboratory analyses

Samples of roughage, concentrate, orts, and feces were dried in a forced oven at 55°C for 72 hours and ground in a mill with a 1-mm screen to determine DM, CP, EE (AOAC, 1990), NDF, ADF, and lignin (Van Soest et al., 1991). Nonfibrous carbohydrates were calculated according to Hall (2000) while total digestible nutrients were calculated according to the equation proposed by Weiss et al. (1992). For analysis of iDM in samples from concentrate, orts, and fecal composite samples, an *in situ* digestibility procedure

was used over the course of 240 h, based on information from Cochran et al. (1986) and Casali et al. (2008). Estimates of fecal excretion were obtained for the equation proposed by Smith and Reid (1955). The determination of the chromium concentration was performed as described by the methodology of Willians et al. (1962).

Chemical composition of milk were determined through the Bentley-2000 infrared analyzer and SCC was determined by the Somacount-500. To determine the values of milk acidity and density, the Dornik solution and thermolactodensimeter were used, respectively (AOAC, 1984). For the determination of methyl esters of FA in milk fat, the milk fat was extracted by centrifugation (17.800×g). The transesterification of triglycerides to obtain FA methyl esters was performed according to the 5509 method of ISO (1978), in n-heptane and KOH/methanol solution. The FA methyl esters were analyzed by gas chromatography Trace-GC Ultra (Thermo Scientific, Tewksbury, MA, USA) equipped with an automatic sampler, flame ionization detector, and a fused silica capillary column (100 m long, 0.25 mm internal diameter, and 0.20 µm - Restek Rt-2560 phase 100% bicianopropil polysiloxane). The gas output was 1.4 mL/min, the flow for the carrier gas (H₂) and for the auxiliary gas (N₂) were 35 mL/minute, and 350 mL/min for synthetic air. The initial temperature of the column was 65°C for 4 minutes, after which it was raised at a rate of 16°C/minute up to 185°C; this lasted for 12 min, then at a rate of 20°C/min, the final temperature of 285°C was reached and maintained for 14 min. The injection volume was 1 microliter and the sample split ratio was 1:100. The peaks were identified through comparisons with the retention times of the standard FA methyl esters (Sigma, St. Louis, MO, USA).

Analyses of butter texture (firmness and adhesiveness) were performed in samples stored at 4°C by using the texture measurer TAXT plus Texture Analyzer (Stable Micro Systems, London, UK) with a conical probe of 45°. The probe penetrated 23 mm into the sample surface at a speed of 3 mm/s; the penetration force applied on the sample indicated the firmness of the butter, and the negative force applied to withdraw the probe indicated the sample's tackiness stickiness.

Statistical analyses

Statistical analyses were performed using the PROC MIXED procedure (SAS Institute, Inc, 2011) with a 2×2 factorial arrangement. The statistic model was as follows: $Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$, where Y_{ijk} = observation of k replication for i treatment in the j period; μ = general mean; T_i = i treatment effect (CG, CGM, CGP, and CMP); P_j = j period effect (1, 2, 3, and 4); A_k = animal effect (1, 2, 3, and 4); and e_{ijk} = random error associated with animal k receiving treatment i in the period j . Treatments were

compared to provide factorial contrasts: pelleted vs non pelleted, with monensin *versus* without monensin, and the interaction between pelleting and monensin. When an interaction was observed, the means were compared using the Scheffe Test. We considered 5% ($p < 0.05$) to be the significance level, and up to 10% ($p < 0.10$) as a tendency.

RESULTS

The DMI of total diet (roughage and concentrate; kg/d) was 12.7 ± 0.83 kg/d (Table 2); being 5.4 ± 0.6 kg was from the concentrate, and 7.8 ± 0.6 kg was from the roughage (data not shown). The roughage:concentrate ratio during all experimental periods was 57:43. Considering the amount of monensin added to the concentrate (31.5 mg/kg DM) and the DMI of concentrate, the daily monensin intake was 169.7 mg/animal. At this amount, monensin did not alter the nutrient intake. However, EE intake was reduced by the pelleting ($p < 0.05$).

We did not observe an interaction between the use of pelleting and the addition of monensin on apparent nutrient digestibility (Table 2). The digestibility was also not influenced by treatment singly, except for apparent ADF digestibility ($p < 0.05$), where pelleting decreased the digestibility by 11.0%.

Milk production and composition were not altered by the addition of monensin or the pelleting. Also, interaction was not observed between the pelleting and the addition of monensin (Table 3). We did not observe treatment interactions on the concentration of PUFA, monounsaturated fatty acids (MUFA), SFA, omega 6, and omega 3 in the milk or for PUFA/SFA and omega 6/omega 3 ratios (Table 4). The pelleting increased MUFA concentration in the milk by 7.4% ($p < 0.05$) and decreased SFA concentration from 54.4 g/100 g to 51.0 g/100 g FA, which corresponds with a reduction of 6.1% ($p < 0.05$); also, the PUFA/SFA ratio ($p < 0.05$) increased and omega 6/omega 3 FA ratios were also increased by 29.5%. Compared to treatments without monensin, the addition of monensin increased PUFA concentration in the milk by 15.3% ($p < 0.05$) and increased omega 6 concentration by 13.0%. Also, the PUFA/SFA ratio ($p < 0.05$) was increased by 21.0%. Saturated fatty acid concentration tended to decrease with the addition of monensin ($p < 0.10$).

Considering FA concentration individually (Table 4), there was an interaction ($p < 0.05$) between the pelleting and the addition of monensin on C6:0, C8:0, C17:0, C17:1, C20:1, and CLA (C18:2 c9 t11) concentrations; the combination of treatments increased the concentration of CLA by 46.9% in the milk when compared to the control treatment. The concentrations of C8:0 and C20:1 were greater with the addition of monensin, while the concentrations of C17:0 and C17:1 were greater with the

Table 2. Nutrient intake (concentrate+pasture) and whole diet (concentrate+pasture) total tract apparent digestibility (TTAD) of Holstein cows grazing on *Cynodon* pasture and supplemented with pelleted or non-pelleted concentrate containing canola seed and with or without monensin addition

Items	Non-pelleted		Pelleted ¹		SE	Probability ²		
	CG	CGM	CGP	CMP		M	P	I
Intake (kg/d)								
DM	13.42	13.02	12.90	11.51	0.84	0.31	0.25	0.57
CP	2.11	2.03	2.06	1.84	0.14	0.30	0.40	0.63
EE	1.05	1.06	0.80	0.83	0.06	0.74	0.03	0.82
NDF	5.76	5.60	6.10	4.95	0.46	0.19	0.75	0.31
ADF	2.77	2.72	2.91	2.32	0.22	0.19	0.56	0.26
NFC ³	3.86	3.65	3.24	3.31	0.29	0.82	0.13	0.65
TTAD (g/kg)								
DM	616.5	606.7	619.4	614.1	0.79	0.36	0.53	0.78
CP	666.3	670.0	699.4	669.6	1.09	0.27	0.17	0.15
EE	873.8	882.5	886.9	909.2	1.15	0.21	0.12	0.57
NDF	485.8	472.8	491.9	460.8	1.89	0.27	0.88	0.64
ADF	422.0	396.3	368.3	359.2	1.94	0.39	0.04	0.68
NFC	782.3	751.9	789.3	769.1	2.68	0.37	0.66	0.85
TDN (g/kg) ³	698.0	685.5	673.3	678.9	1.11	0.76	0.19	0.44

SE, standard error; DM, Dry matter; CP, Crude protein; EE, Ether extract; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; NFC, non fibrous carbohydrates; TDN, total digestive nutrients.

¹ CG, feed concentrate with ground canola seeds; CGM, feed concentrate CG with sodium monensin addition; CGP, feed concentrate CG pelleted; CMP, feed concentrate CG with sodium monensin addition and pelleted.

² M, monensin effect; P, pelleted effect; I, interaction effect.

³ $NFC = 100 - (CP\% + (NDF\% - NDF_{cp}) + EE\% + MM\%)$ (Hall, 2000); $TDN = CPD\% + NDFD\% + NFCD\% + (EED\% \times 2.25)$ (Weiss et al., 1992), were NDF_{cp} , neutral detergent fiber corrected for crude protein; MM, mineral matter; CPD, crude protein digestible; NDFD, Neutral detergent fiber digestible; NFCD, non fibrous carbohydrates digestible; EED, ether extract digestible.

pelleting treatment. There was an effect of monensin ($p < 0.05$) on C4:0 and C18:0 concentrations, as they were reduced by the addition of monensin by 18.0% and 9.2%, respectively; meanwhile, the concentrations of C16:1 n-7, C18:2 n-6, and C20:3 n-6 were increased. The pelleting decreased ($p < 0.05$) the concentrations of C10:0, C14:0, and C18:0 by 28.0%, 13.5%, and 15.0%, respectively; however, the pelleting increased the concentrations of C16:1 n-7, C16:1 n-9, and C18:1 n-9. Although PUFA concentration

increased and SFA concentration decreased in the milk with the addition of monensin and the pelleting; the treatments did not influence the texture of the butter (Table 5).

DISCUSSION

The results of the influence of monensin on DMI are quite varied, thus showing that they are different according to the amount of monensin supplied and among cows

Table 3. Production and milk composition of Holstein cows grazing on *Cynodon* pasture and supplemented with pelleted or non-pelleted concentrate containing canola seed and with or without monensin addition

Items	No-pelleted		Pelleted ¹		SE	Probability ²		
	CG	CGM	CGP	CMP		P	M	I
Milk production (kg/d)	16.36	14.12	13.12	15.27	1.4	0.48	0.97	0.15
Fat (g/kg)	38.5	34.7	31.3	32.6	0.26	0.11	0.64	0.35
Protein (g/kg)	29.7	28.7	31.1	28.8	0.15	0.62	0.32	0.68
Lactose (g/kg)	42.1	44.6	44.1	4.30	0.11	0.85	0.56	0.13
Total solids (g/kg)	118.5	115.8	115.0	112.4	0.23	0.17	0.28	0.99
SCC (log ₁₀)	2.13	2.00	2.23	2.19	0.24	0.56	0.75	0.84
Urea-N (mg/dL)	13.14	12.39	12.53	13.09	0.90	0.96	0.92	0.49
Acidity (°D)	19.6	19.7	19.2	18.9	0.05	0.26	0.83	0.72
Density (g/L)	1,027	1,027	1,027	1027	0.73	0.90	0.85	0.91

SE, standard error; SCC, somatic cell count.

¹ CG, feed concentrate with ground canola seeds; CGM, feed concentrate CG with sodium monensin addition; CGP, feed concentrate CG pelleted, CMP, feed concentrate CG with sodium monensin addition and pelleted.

² P, pelleted effect; M, monensin effect; I, interaction effect.

Table 4. Lipid composition (g/100 g of fatty acids) of milk of Holstein cows grazing on *Cynodon* pasture and supplemented with pelleted or non-pelleted concentrate containing canola seed and with or without monensin addition

Fatty acids	No-pelleted		Pelleted ¹		SE	Probability ²		
	CG	CGM	CGP	CMP		M	P	I
C4:0	0.79	0.72	0.79	0.57	0.06	0.04	0.26	0.30
C6:0	0.75	0.8	0.51	1.87	0.14	<0.01	0.02	<0.01
C8:0	0.30	0.59	0.50	0.39	0.08	0.29	1.00	0.04
C10:0	1.04	1.15	0.61	0.95	0.12	0.09	0.03	0.38
C12:0	1.21	1.16	1.04	1.10	0.14	0.95	0.43	0.70
C13:0	1.37	1.34	0.94	1.38	0.14	0.18	0.19	0.12
C14:0	5.05	5.28	4.37	4.63	0.28	0.39	0.04	0.95
C14:1n5	0.31	0.32	0.31	0.31	0.02	0.71	0.88	0.99
C14:1n7	0.45	0.47	0.42	0.50	0.03	0.16	0.97	0.51
C15:0	0.67	0.81	0.73	0.83	0.09	0.21	0.61	0.81
C16:0	18.13	18.35	20.03	18.47	0.49	0.21	0.07	0.10
C16:1n7	0.16	0.18	0.17	0.27	0.02	0.03	0.05	0.13
C16:1n9	0.51	0.58	0.66	0.69	0.03	0.12	<0.01	0.54
C17:0	0.59	0.60	0.76	0.59	0.02	<0.01	<0.01	<0.01
C17:1	0.38	0.32	0.64	0.33	0.02	<0.01	<0.01	<0.01
C18:0	23.93	22.62	21.24	18.41	0.66	0.01	<0.01	0.28
C18:1n9	38.45	38.10	40.31	41.59	0.51	0.39	<0.01	0.15
C18:2 c9 tr11	0.96	0.90	0.86	1.41	0.13	0.09	0.14	0.04
C18:2n6	3.31	3.79	3.65	4.04	0.18	0.04	0.15	0.79
C18:3n3	0.47	0.48	0.36	0.46	0.06	0.34	0.28	0.44
C20:0	0.43	0.55	0.32	0.49	0.08	0.09	0.30	0.74
C20:1	0.16	0.20	0.18	0.16	0.01	0.45	0.50	0.04
C20:3n6	0.04	0.10	0.09	0.12	0.02	0.05	0.13	0.57
C20:4n6	0.15	0.15	0.14	0.13	0.01	0.68	0.19	0.89
C22:2	0.13	0.16	0.14	0.13	0.02	0.58	0.64	0.22
C23:0	0.26	0.22	0.23	0.18	0.04	0.23	0.35	0.87
PUFA	5.06	5.58	5.24	6.29	0.24	0.01	0.10	0.32
MUFA	40.42	40.17	42.69	43.85	0.51	0.40	<0.01	0.20
SFA	54.52	54.25	52.07	49.86	0.64	0.09	<0.01	0.17
Omega 6	3.50	4.04	3.88	4.29	0.18	0.03	0.12	0.72
Omega 3	0.47	0.48	0.36	0.46	0.06	0.34	0.28	0.44
PUFA/SFA	0.09	0.10	0.10	0.13	0.006	0.01	0.02	0.21
Omega 6/omega 3	7.58	8.58	10.85	10.06	1.03	0.92	0.05	0.41

SE, standard error; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

¹ CG, feed concentrate with ground canola seeds; CGM, feed concentrate CG with sodium monensin addition; CGP, feed concentrate CG pelleted; CMP, feed concentrate CG with sodium monensin addition and pelleted.

² M, monensin effect; P, pelleted effect; I, interaction effect.

confined, on pasture or receiving diets high in roughage (Ruiz et al., 2001; Ipharraguerre and Clark, 2003; Gandra et al., 2010). The mode of action of monensin in reducing DMI in dairy cows can be mediated by an increase in propionate concentration in the rumen, which would meet the glucose demand of the mammary gland by

Table 5. Butter texture of milk produced by cows grazing on pasture and supplemented with pelleted concentrate or not, with or without addition of monensin butter

Texture (g)	No-pelleted		Pelleted ¹		SE	Probability ²		
	CG	CGM	CGP	CMP		M	P	I
Adhesiveness	-7.408	-7.170	-7.199	-6.880	0.86	0.75	0.78	0.96
Firmness	37.202	44.383	40.442	39.401	3.06	0.34	0.78	0.21

SE, standard error.

¹ CG, feed concentrate with ground canola seeds; CGM, feed concentrate CG with sodium monensin addition; CGP, feed concentrate CG pelleted; CMP, feed concentrate CG with sodium monensin addition and pelleted.

² M, monensin effect; P, pelleted effect; I, interaction effect.

gluconeogenesis. Thus, when the demand for glucose by the body is reduced, the excess propionate can be oxidized in the liver by reducing starvation (Oba and Allen, 2003). Grazing cattle or those feeding high amounts of roughage increases the difficulty of meeting the energy demand (NRC, 2001). When monensin is added to the diet of grazing animals, the greater propionate production and reduced methane production yield more energy to the animal without affecting consumption (Ipharraguerre and Clark, 2003). Reduction in EE consumption using pelleting can be explained by how the pelleting reduces the EE content in the concentrate (data not show) and in the total diets (Table 1). It is common to have oil loss during the thermal processing of foods (Riaz, 2000) and what leads us to consider this hypothesis is that we performed only one mixture of the ration, which a portion of this mixture was pelleted and another not, thereby constituting the treatment CGP and CG, respectively. Therefore, the CGP and CG treatments had the same composition, with the heat treatment as the only difference between them. A reduction in EE intake in the total diet was also observed by dos Santos et al. (2011) that worked with ground sunflower seeds in a pelleted vs non-pelleted concentrate treated with or without lignosulfonate.

The pelleting reduced the ADF contents of the concentrate (data not show), as well as reducing the intake of these nutrients. This effect also was observed in the fiber digestibility (Table 2). One hypothesis would be the negative interference from high levels of EE in the samples during the measurement by gravimetric difference of the fiber, because the EE affects the filter process, and in some situations, promotes the obstruction of the equipment due to an excess of fat in the sample (AOAC, 1997). Except for the lower apparent digestibility of ADF by the pelleting, our results agree with other studies in which the canola seeds thermally protected did not affect the digestibility of nutrients (Neves et al., 2009). Moreover, similar results were observed by Neves et al. (2007), where the authors observed a reduction in digestibility of NDF and ADF in diets that were thermally treated.

The increase in the hepatic flow of gluconeogenesis precursors caused by the addition of monensin can promote an average increase of 2% in the milk production of dairy cows (Duffield et al., 2008). In the present study, monensin was given to the primiparous cows over the course of 135 days of lactation. The monensin concentration in the diet was 31.5 g/kg (intake of 169.7 mg/d), which was much less than that provided in other experiments (above 320 mg monensin/d) where the production or milk composition, mainly fat, were altered. The differences between the results of some studies also are influenced by the stage of lactation and feeding (Ruiz et al., 2001; da Silva et al., 2007). In grazing animals or those fed high roughage levels, the

major beneficial effect of monensin is the greater production of propionic acid vs methane production and acetic acid, thereby resulting in an improved energy efficiency due to the inhibitory effect of monensin on cellulolytic gram-positive bacteria, and a consequent increase in milk production and leading to milk fat depression (Duffield et al., 2008). Monensin has shown a greater benefit in the performance of grazing cattle, or cattle on diets containing high levels of roughage, than diets with high concentrate levels (Lana and Russell, 2001). Although the animals in the present study were kept on pasture, monensin did not alter the production and milk composition. One factor that could have prevented the effect of monensin in this study was the addition of sodium bicarbonate to the concentrate, because it is a buffering agent that increases the proportion of acetate/propionate in the rumen; in other words, it has the opposite effect of monensin (Nagajara et al., 1997). Another issue is the number of animals used in the present work that can have contributed with the results.

In the recent past of human nutrition, some fatty acids, mainly the lauric acid (C12:0), myristic (C14:0), and palmitic acid (C16:0) were considered to be atherogenic (Mensink et al., 2003). In these sense, the results observed in this study were positive for myristic acid in pelleted treatments, since pelleting reduced ($p < 0.05$) the content of this fatty acid in milk by 13.5%. Despite of the tendency to increased levels of palmitic acid ($p < 0.10$) in pelleted treatments, studies in rats show that palmitic acid has reduced atherogenic effects when consumed along with adequate levels of unsaturated FA as linoleic acid or omega-6 (1/3 of total fatty acids, Clandinin et al., 2000; Fernandez and Webb, 2008), this fatty acid is in canola, and its concentration increased in milk with the addition of monensin (Table 3). Indeed, recent meta-analysis showed that there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of coronary heart disease or cardiovascular disease (Schwingshackl and Hofmann, 2014).

The analysis of 10 experiments using monensin showed a decrease in the synthesis of short and medium chains of FA and of stearic acid (C18:0), but an increase in the synthesis of long chain FA such as CLA (Duffield et al., 2008). Similar results were observed in the present study, where monensin decreased C18:0 by 9.2% and tended to increase the CLA content by 26.9%. Besides monensin, the pelleting also reduced the concentration of C18:0 by 15.0% (23.28 for non-pelleted vs 19.83 g/100 g of FA for pelleted concentrates), thus agreeing with dos Santos et al. (2011). The reduced concentration of C18:0 may indicate a decrease in the ruminal biohydrogenation process (final product of ruminal biohydrogenation) and that more intermediary products, such as the C18:1 trans-11 (vaccenic acid), were retained in the rumen and subsequently

converted to CLA by the delta-9-desaturase in the mammary gland.

Both the pelleting and monensin promoted an increase in palmitoleic acid (C16:1 n-7) production, and although numerically higher, the pelleting and monensin had no interaction effect. The palmitoleic acid is a fatty acid omega-7, which is abundant in plants; also, it is biosynthesized from palmitic acid (C16:0) by the action of the enzyme delta-9 desaturase (Yang et al., 2011). Recent studies in mice and humans show that the fatty acid C16:1n7 improves blood lipid composition and increases insulin sensitivity by inhibiting apoptosis of pancreatic beta cells (Yang et al., 2011). In the present study there was no influence of treatments on blood parameters in the animals (unpublish data).

The pelleting was effective in protecting the oleic acid (C18:1 n-9), thereby increasing its concentration in milk by 6.9%. The C18:1 n-9 occurs naturally in the fat from animals and vegetables; in canola it represents 53% to 70% of FA (ANVISA, 1999). The literature does not mention C18:1 n-9 as one of the intermediates of fatty acid biohydrogenation of C18:2 c9 c12 (linoleic acid - omega 6) and C18:3 c9 c12 c15 (α -linolenic acid, omega 3; Shingfield et al., 2010). The use of PUFA sources and heat treatment, such as extrusion, led to an increased CLA concentration (Akraim et al., 2007). In this study, the pelleting alone did not occur, probably due to the fact that the temperature in the pelleting was lower than in the extrusion process. However, the pelleting combined with monensin were effective in increasing the CLA levels by 46.9% ($p < 0.05$). This result corroborates with some studies that reported an increase in CLA concentration in diets that were thermally treated or contained added monensin (Neves et al., 2009; da Silva-Kazama et al., 2011).

The omega 3 and 6 FA compete in the organism through metabolism by the enzyme delta-6-desaturase metabolism. Thus, the imbalance in the ratio of these FA contributes to the occurrence of diseases such as cancer, Alzheimer's, obesity, and inflammatory processes, such that omega-6 increases the inflammatory processes while omega 3 decreases them (Petit, 2010). While a ratio of omega-6/omega-3 of 10 to 5:1 may be considered satisfactory (Nutrition and Health Collection 1999; Institute of Medicine, 2002), the most recent proposal, based on animal experiments, is 1:1 (Fürst, 2002). In this study, the average ratio (10.5 g/100 g of FA) was above the recommended ratio.

The SFA content in milk is strongly correlated with the firmness of butter (Hurtaud et al., 2010). Differences in the firmness of butter occur, for example, when there is a change in the pastures grazed during summer versus silage intake during winter, which is directly related to the change in fatty acid composition of milk fat (Couvreux et al., 2006).

Considering that the process of butter production does not alter the fatty acid composition (da Silva-Kazama et al., 2010; Hurtaud et al., 2010; Oeffner et al., 2013), we expected an improvement of the butter texture and spreadability. However, the changes in the fatty acid profile were not sufficient to yield those improvements.

CONCLUSION

Supplying monensin to cows kept on pasture and supplementing them with concentrate containing ground canola seeds does not alter the intake and digestibility. Pelleting reduces EE and ADF intake, and also reduces the ADF digestibility of concentrates containing canola seeds. The association of pelleting with monensin does not alter the production, milk composition, and butter texture. However, it alters the lipid composition of milk. Pelleting increases the omega 6:omega 3 ratio of milk, and the pelleting associated with monensin increases the CLA concentration in milk.

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