

## Pelleting and sodium monensin increase milk CLA concentration from dairy cows fed flaxseed<sup>¶</sup>

*El peletizado y la monensina sódica incrementan la concentración de CLA en leche de vacas suplementadas con linaza*

*Peletização e monensina sódica aumentam a concentração de CLA no leite de vacas alimentadas com linhaça*

Jakeline Vieira Romero<sup>1</sup>, Zoot, PhD; Francilaine Eloise De Marchi<sup>2</sup>, Zoot, PhD; Claudete Regina Alcalde<sup>2</sup>, Zoot, PhD; Ulysses Cecato<sup>2</sup>, Zoot, PhD; Paula Adriana Grande<sup>2</sup>, Zoot, PhD; Altair Antônio Valloto<sup>3</sup>, MV, Esp.; Daniele Cristina Silva-Kazama<sup>4</sup>, Zoot, PhD; Geraldo Tadeu dos Santos<sup>2\*</sup>, MV, PhD.

<sup>1</sup>Faculdade de Medicina Veterinária, Universidade de Cuiabá, Cuiabá-MT, Brazil.

<sup>2</sup>Pós-Graduação em Zootecnia, Departamento de Zootecnia, Universidade Estadual de Maringá – UEM, Paraná, Brazil.

<sup>3</sup>Associação Paranaense dos Criadores de Bovinos da Raça Holandesa, Curitiba-PR, Brazil.

<sup>4</sup>Universidade Federal de Santa Catarina, Florianópolis-SC, Brazil.

(Received: August 24, 2016; accepted: July 25, 2017)

doi: 10.17533/udea.rccp.v31n2a04

### Abstract

**Background:** Demand for naturally enriched foods is increasing worldwide. Animal nutrition allows incorporating nutraceutical molecules into milk. These molecules are intended for improving human health. **Objective:** To evaluate the effects of pelleting and monensin addition to the diet on intake, digestibility of dry matter (DM) and nutrients, milk yield and composition, and fatty acids (FA) profile in cows kept on pasture and supplemented with concentrate containing ground flaxseed (GF). **Methods:** Four Holstein cows were assigned to the following treatments: 1) concentrate with GF; 2) GF + 32.93 mg/Kg monensin (GFM); 3) GF pelleted (GFP); 4) GF pelleted + 32.93 mg/Kg monensin (FMP). **Results:** Treatments did not show effects on digestibility of dry matter (DM) and nutrients or feed intake, with the exception of ether extract intake, which was reduced by 28% with pelleting. Addition of monensin increased the conjugated linoleic acid (CLA) concentration by 47%. Pelleting increased the concentrations of CLA (70%), polyunsaturated FA (PUFA; 25%),

¶ To cite this article: Vieira Romero J, De Marchi FE, Alcalde CR, Cecato U, Grande PA, Valloto AA, Silva-Kazama DC, dos Santos GT. Pelleting and sodium monensin increase milk CLA concentration from dairy cows fed flaxseed. Rev Colomb Cienc Pecu 2018; 31(2):110-119.

\* Corresponding author: Geraldo Tadeu dos Santos. Pós-Graduação em Zootecnia, Departamento de Zootecnia, Universidade Estadual de Maringá – UEM, Paraná, Brazil 87020-900. Tel.: +55-44-3011-8947. Fax: +55-44-3011-8977. E-mail: gtsantos@uem.br

and monounsaturated FA (MUFA; 16%), and reduced the concentrations of steric acid (C18) in 19% and total saturated FA (SFA; 14%). **Conclusion:** Pelleting and monensin does not alter feed intake and digestibility of DM and nutrients. Pelleting of concentrate feed containing flaxseed improves milk FA quality.

Keywords: *biohydrogenation, ionophore, linoleic acid, Linum usitatissimum, polyunsaturated fatty acids.*

## Resumen

**Antecedentes:** La demanda por alimentos naturalmente enriquecidos se ha venido incrementado en todo el mundo. A través de la nutrición animal es posible incorporar moléculas nutraceuticas en la leche. El uso de estas moléculas pretende mejorar la salud humana. **Objetivo:** Evaluar los efectos de la peletización y la monensina sobre la ingesta, digestibilidad de la materia seca (DM) y nutrientes, producción de leche, composición y perfil de ácidos grasos (FA) en leche de vacas en pastoreo que consumen alimento concentrado formulado con linaza molida (GF). **Métodos:** Cuatro vacas Holstein fueron distribuidas en los siguientes tratamientos: 1) Ración conteniendo GF; 2) GF + 32,93 mg/Kg de monensina (GFM); 3) GF en pellets (GFP); 4) GF en pellets + 32,93 mg/Kg de monensina (FMP). **Resultados:** Los tratamientos no mostraron efectos en el consumo y digestibilidad de la DM y nutrientes, excepto por el consumo de extracto etéreo, con una reducción del 28% en las raciones peletizadas. La monensina incrementó la concentración de ácido linoleico conjugado (CLA) en 47%. La peletización aumentó el contenido de CLA (70%), FA poliinsaturados (PUFA; 25%) y FA monoinsaturados (16%); y redujo las concentraciones de C18 en 19%, y FA saturados total en 14%. **Conclusión:** El peletizado y la monensina no alteran la ingesta de alimento ni la digestibilidad de la DM y nutrientes. El proceso de peletizado aplicado a concentrados que contienen linaza mejora la calidad de los ácidos grasos de la leche.

**Palabras clave:** *ácido linoleico, ácidos grasos poliinsaturados, biohidrogenación, ionóforo, Linum usitatissimum.*

## Resumo

**Antecedentes:** A procura por alimentos naturalmente enriquecidos tem aumentado por parte dos consumidores. Através da nutrição animal é possível incorporar ao leite moléculas nutraceuticas, essas moléculas são conhecidas por melhorarem a saúde humana. **Objetivo:** Avaliar os efeitos da peletização e monensina sobre o consumo, digestibilidade da matéria seca (DM) e nutrientes, produção de leite, composição e perfil de ácidos graxos (FA) no leite de vacas em pastejo e suplementadas com concentrado contendo linhaça moída (GF). **Métodos:** quatro vacas Holandesas foram distribuídas nos tratamentos: 1) concentrado contendo GF; 2) GF + 32,93 mg/Kg de monensina (GFM); 3) GF peletizado (GFP); 4) GF peletizado + 32,93 mg/Kg de monensina (FMP). **Resultados:** os tratamentos não apresentaram efeitos sobre consumo e digestibilidade da DM e dos nutrientes, exceto para o consumo de extrato etéreo, com redução de 28% com a peletização do concentrado. A adição de monensina aumentou a concentração de ácido linoleico conjugado (CLA) em 47%. A peletização aumentou os teores de CLA (70%), FA poli-insaturados (25%) e FA monoinsaturados (16%); e reduziu as concentrações de C18 em 19% e AG saturados totais em 14%. **Conclusão:** Peletização e monensina na alteram o consumo e digestibilidade da DM e dos nutrientes. O processo de peletização aplicado em concentrados contendo linhaça melhora a qualidade dos FA do leite.

**Palavras chave:** *ácido linoleico, ácidos graxos poli-insaturados, biohidrogenação, ionóforo, Linum usitatissimum.*

## Introduction

Two groups of polyunsaturated fatty acids (PUFA), linolenic (C18:3) and linoleic fatty acids (FA) (C18:2) have been recognized as very important for animal nutrition and human health. Flaxseed (*Linum usitatissimum*) has high concentrations of antioxidants and alfa-linolenic FA (C18:3n3), this PUFA represents approximately 53% of the total FA present in flaxseed

(Petit and Côrtes, 2010). PUFA-rich seeds have been fed to dairy cows to improve milk fat quality by increasing its PUFA concentrations, mainly omega 3 FA. However, ruminal biohydrogenation transforms PUFA into saturated FA (SFA). Ruminal biohydrogenation is not entirely bad for ruminant products; some intermediates in this process have been shown beneficial for human health, such as C18:2 c9 t11 CLA (Shingfield *et al.*, 2010). Therefore,

it is necessary to control ruminal biohydrogenation to improve milk fat quality. The main FA of CLA family, C18:2 c9 t11, is the first intermediate of the ruminal biohydrogenation of C18:2, released by *Butyrivibrio fibrisolvens*. Furthermore, CLA can be synthesized in the mammary gland from C18:1 t11, another intermediate of incomplete biohydrogenation of unsaturated FA (Shingfield *et al.*, 2010; Crumb and Vatter, 2011). According to several studies, these FA are beneficial for human health because they have anticarcinogenic and antiatherogenic properties, and improve immune response (Shingfield *et al.*, 2010; Crumb and Vatter, 2011).

Heat treatment, such as extrusion and pelleting, has positive effects, by reducing the extent of biohydrogenation and preserving some PUFA (Gómez-Cortés *et al.*, 2009; Neves *et al.*, 2009; Dos Santos *et al.*, 2011). Further, ionophores can transform milk FA; monensin increases C18:1 concentration and reduction of C10, C12, C16, and 18:0 (FA of the end of biohydrogenation). Consequently, higher amounts of CLA may be formed in the rumen or in the mammary gland (Da Silva *et al.*, 2007; Da Silva-Kazama *et al.*, 2010). The aim of the present study was to evaluate the effects of pelleting and monensin addition on feed intake, digestibility of DM and nutrients, milk production, milk composition, and milk fat profile in Holstein cows kept on pasture and supplemented with concentrate containing ground flaxseed.

## Materials and methods

### Ethical considerations

All animal procedures were approved by the Animal Care and Use Committee of Universidade Estadual de Maringá (UEM), Brazil (Protocol No. 021/2008 - 036/2008).

### Animals and diets

Four primiparous Holstein cows averaging  $186 \pm 10$  d in milk and  $515 \pm 36$  Kg body weight at the beginning of the experiment were used in a Latin square design with a  $2 \times 2$  factorial arrangement of treatments and four 21-d periods (16 d for acclimation and 5 d for collections). The animals were kept on

rotational grazing with free access to water. Cows were milked twice daily (06:00 and 15:00 h), and were subsequently housed in a tie stall for approximately 3 h to receive the concentrate feed. All diets contained ground flaxseed. The difference between treatments was the addition of sodium monensin and the pelleted versus no monensin addition and non-pelleted concentrate feed (Table 1). The concentrate feeds were balanced to provide isonitrogenous and isocaloric diets to meet the requirements of Holstein cows, according to NRC (2001). The pasture area consisted of four paddocks (0.4 Ha each) with *Cynodon* grass. Animals were placed in the paddocks when the grass was 40 cm high on average, and removed from them when the grass was about 20 cm high (Corrêa and Santos, 2003; Paris *et al.*, 2016).

**Table 1.** Ingredients and chemical composition of concentrate feeds.

	Non pelleted		Pelleted <sup>1</sup>	
	GF	GFM	GFP	FMP
<i>Ingredients (g/Kg of DM)</i>				
Ground corn	671.4	671.4	671.4	671.4
Soybean meal	85.7	85.7	85.7	85.7
Ground flaxseed	142.8	142.8	142.8	142.8
Limestone	8.5	8.5	8.5	8.5
Calcium phosphate	4.6	4.6	4.6	4.6
Salt	1.1	1.1	1.1	1.1
Mineral mix <sup>2</sup>	68.6	68.6	68.6	68.6
Sodium bicarbonate	17.4	17.4	17.4	17.4
Sodium monensin (mg/Kg)	-	32.93	-	32.93
<i>Chemical composition (g/Kg DM)</i>				
Dry matter (g/Kg natural matter)	910.2	907.9	896.2	892
Crude protein	137	136.4	138	140.3
Ether extract	75.1	74.4	43.5	58.2
Neutral detergent fiber	106	105.1	114	105.2
Acid detergent fiber	49.4	46.8	49.6	48.9

<sup>1</sup>GF: Concentrate with ground flaxseed. GFM: GF + monensin. GFP: GF pelleted. FMP: GF pelleted + monensin. <sup>2</sup>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 IU/Kg; Vit. D = 68,000 IU/Kg; Vit. E = 450 mg/Kg.

To determine pasture composition, samples of grazed stratum were manually collected from several points in each paddock. Grass contained on average  $270 \pm 15$  g/Kg DM,  $164 \pm 25$  g/Kg crude protein (CP),  $22 \pm 3$  g/Kg ether extract (EE),  $665 \pm 46$  g/Kg neutral

detergent fiber (NDF),  $315 \pm 26$  g/Kg acid detergent fiber (ADF), and  $28 \pm 1$  g/Kg lignin. The available forage mass was 1.7 ton DM/Ha.

#### *Experimental procedures and chemical analysis*

The experiment was conducted during the wet season (December to February). Average precipitation and temperature were 175 mm and 26 °C, respectively during the trial (Maringá Main Climatological Station, located at UEM).

To estimate dry matter intake (DMI) and nutrient intake of concentrate feeds, on the last five days of each experimental period, concentrate andorts were sampled and stored at -20 °C for further analysis of DM, CP, EE, NDF and ADF. Roughage intake was estimated using indigestible dry matter (iDM) as internal marker, based on Cochran *et al.* (1986) and Casali *et al.* (2008). Indigestible DM was estimated in the samples of concentrates, orts, and feces by *in situ* digestibility during 240 h. Fecal excretion was estimated with chromium oxide ( $\text{Cr}_2\text{O}_3$ ) as external marker. The  $\text{Cr}_2\text{O}_3$  was provided orally in cellulose bags, twice daily (5 g for each animal), from the 5<sup>th</sup> to the 15<sup>th</sup> day of each experimental period (Detmann *et al.* 2001). Fecal samples were collected twice daily (08:00 and 16:00 h) from the rectum, between the 10<sup>th</sup> and 15<sup>th</sup> day, and a composite sample was proportionally collected on a dry weight basis per animal/period for further analyses. Samples of roughage, concentrate, orts, and feces were dried in a forced oven at 55 °C for 72 h and ground in a mill through a 1 mm screen to determine DM, CP, EE, NDF, ADF, and lignin.

Milk production was recorded daily and fat-corrected milk (FCM; 3.5%) was calculated as follows:  $\text{FCM} = (0.432 + 0.1625 \times \text{percentage of milk fat}) \times \text{Kg milk/d}$  (Sklan *et al.*, 1992). Milk was sampled on the 17<sup>th</sup> and 18<sup>th</sup> day of each experimental period, and a composite sample was proportionately collected in relation to milk production. The sample was then stored in a plastic bottle and preserved with 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 at -20 °C for lipid composition analysis. Milk composition was determined through a Bentley-2000

infrared analyzer and SCC was determined using a Somacount-500. Milk fat was extracted by centrifugation ( $17,800 \times g$ ) for determination of FA methyl esters. 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 in a Trace-GC Ultra equipment (Thermo Scientific, Tewksbury, MA, USA) equipped with automatic sampler, flame ionization detector, and a fused silica capillary column (100 m long, 0.25 mm internal diameter, and 0.20  $\mu\text{m}$  - Restek Rt-2560 phase 100% bicianopropil polysiloxane). Gas output was 1.4 mL/min. Flow for the carrier gas ( $\text{H}_2$ ) and for the auxiliary gas ( $\text{N}_2$ ) was 35 mL/min, and 350 mL/min for synthetic air. Initial temperature of the column was 65 °C for 4 min, and then raised at a rate of 16 °C/min up to 185 °C, which lasted 12 min; then, at a rate of 20 °C/min. 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).

#### *Statistical analysis*

Statistical analyses were performed with the PROC MIXED procedure [SAS, 2002. Statistical Analysis System, SAS/STAT® user's guide. Version 9.0 (TS Level 00M0) Cary NC, USA] using a 2×2 factorial arrangement. The 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.

$\mu$ : General mean.

$T_i$ :  $i$  treatment effect.

$P_j$ :  $j$  period effect (1, 2, 3, and 4).

$A_k$ : Animal effect (1, 2, 3, and 4).

$e_{ijk}$ : Random error associated with  $k$  animal receiving  $i$  treatment in the  $j$  period.

Treatments were compared to provide factorial contrasts: pelleted *versus* non pelleted, with monensin

versus without monensin, and the interaction between pelleting and monensin. We set the significance level at 5% ( $p < 0.05$ ), and up to 10% ( $p > 0.051 < 0.10$ ) as a tendency.

## Results

Pelleting and monensin did not change milk production and composition (Table 2). Average milk production and milk fat concentration was 11.44 Kg/d and 37 g/Kg, respectively. Milk fat was reduced by monensin supplementation by approximately 8%. Proteins, lactose, total solids, and urea nitrogen content averaged 33.5, 45, 12.5 g/Kg, and 11.2 mg/dL, respectively.

The concentration of 16:1n9, 18:1 t9, 18:2n6, and CLA (C18:2 c9 t11) increased by the pelleting process (Table 3). Indeed, pelleting reduced C18, C20:2, C24 and milk SFA concentration. The concentration of C14 and C20 tended to decrease with the pelleting process. Monensin was effective for increasing C18:1 t9 and CLA content. Stearic acid (C18) was reduced by approximately 18% in the milk of cows fed pelleted diets. The CLA concentration in milk fat increased by approximately 47% by monensin addition. The

CLA concentration was 70% higher in pelleted diets compared to non-pelleted diets.

The addition of monensin did not change PUFA, MUFA, SFA,  $\omega 6$ ,  $\omega 3$ , PUFA/SFA, and  $\omega 6/\omega 3$  ratios. Pelleting increased PUFA concentration by 25% and MUFA by 15%. Furthermore, milk SFA concentration was reduced from 55.17 to 47.38 g/100 g FA by pelleting, corresponding to a 14% reduction. Pelleted diets also increased  $\omega 6$  concentration by 24.5% without changing the  $\omega 3$  concentration in milk. In fact, PUFA/SFA and  $\omega 6/\omega 3$  were 53.8 and 27.8% higher for pelleted diets, respectively. Therefore, the main effect on milk fat composition was observed with pelleted diets.

Nutrient intake was not affected by treatment (Table 4), with the exception of EE intake, where pelleting reduced intake by 28%. Considering the amount of monensin added to the concentrate feed (32.93 mg/Kg MS) and the DMI from concentrate feed (5.4 Kg/d), daily monensin intake was 178 mg/animal. This amount did not change the diet intake of cows. There were no differences on apparent total digestibility of nutrients because of pelleting and monensin.

**Table 2.** Production and milk composition of Holstein cows grazing on *Cynodon* pasture and supplemented with concentrate feed containing flaxseed.

	Non pelleted		Pelleted <sup>1</sup>		SEM	Probability <sup>2</sup>		
	GF	GFM	GFP	FMP		P	M	I
Milk production (Kg/d)	12.55	12.17	9.98	11.14	1.37	0.21	0.76	0.57
Fat (g/Kg)	38.7	37.4	37.9	33.4	0.37	0.53	0.45	0.67
Protein (g/Kg)	33.2	32.8	34.2	33.7	0.14	0.49	0.76	0.94
Lactose (g/Kg)	45.1	45.8	44.3	45.2	0.08	0.38	0.31	0.93
Total solids (g/Kg)	12.60	12.54	12.55	12.15	0.43	0.62	0.60	0.71
Fat (Kg/day)	0.45	0.44	0.34	0.38	0.05	0.10	0.81	0.58
Protein (Kg/d)	0.39	0.38	0.33	0.38	0.04	0.50	0.64	0.51
SCC ( $\log_{10}$ ) <sup>3</sup>	2.79	2.60	2.75	2.57	0.17	0.86	0.32	0.98
Urea-N (mg/dL)	11.68	11.88	10.06	11.05	1.02	0.26	0.57	0.71

<sup>1</sup>GF: Concentrate with ground flaxseeds. GFM: GF + monensin. GFP: GF pelleted. FMP: GF pelleted + monensin. <sup>2</sup>SEM: Standard error of the mean. P: Pelleting effect. M: Monensin effect. I: Interaction effect. <sup>3</sup>SCC: Somatic cell count.



**Table 3.** Lipid composition (g/100 g of FA) of milk from Holstein cows grazing on *Cynodon* pasture and supplemented with concentrate feed containing flaxseed.

Fatty acids	Non pelleted		Pelleted <sup>1</sup>		SEM	Probability <sup>2</sup>		
	GF	GFM	GFP	FMP		P	M	I
4:0	0.72	0.41	0.24	0.39	0.21	0.27	0.71	0.30
6:0	0.69	0.50	0.28	0.39	0.16	0.14	0.82	0.38
8:0	0.46	0.41	0.19	0.31	0.11	0.14	0.79	0.48
10:0	0.99	1.01	0.47	0.77	0.24	0.14	0.52	0.56
11:0	0.10	0.11	0.04	0.08	0.03	0.18	0.40	0.69
12:0	1.18	1.27	0.68	1.04	0.22	0.12	0.32	0.55
13:0	0.08	0.05	0.04	0.07	0.02	0.58	0.70	0.14
14:0	5.35	5.88	3.86	5.02	0.56	0.06	0.16	0.58
14:1n7	0.56	0.63	0.51	0.60	0.04	0.29	0.09	0.82
14:1n5	0.39	0.44	0.31	0.42	0.06	0.46	0.23	0.63
15:0	0.93	1.01	0.87	0.89	0.06	0.18	0.45	0.62
16:0	19.84	21.28	19.70	20.06	1.32	0.62	0.51	0.69
16:1n9	0.55	0.69	0.78	0.83	0.07	0.02	0.20	0.53
16:1n7	0.21	0.31	0.23	0.26	0.04	0.78	0.16	0.42
17:0	0.64	0.73	0.73	0.69	0.07	0.71	0.81	0.38
17:1	0.27	0.31	0.36	0.32	0.05	0.34	0.10	0.42
18:0	22.31	23.02	20.36	16.60	1.30	0.01	0.27	0.12
18:1 tr9	2.00	2.66	3.37	4.68	0.27	<0.01	<0.01	0.27
18:1 c9	35.00	30.98	38.26	35.91	2.56	0.15	0.25	0.75
18:2n6	4.54	4.71	5.40	6.28	0.51	0.04	0.33	0.50
20:0	0.24	0.28	0.24	0.21	0.02	0.07	0.68	0.05
20:1	0.16	0.22	0.16	0.15	0.02	0.10	0.25	0.09
18:3n3	1.08	1.06	0.98	1.08	0.17	0.82	0.81	0.70
C18:2 c9 t11	0.95	1.26	1.46	2.29	0.16	<0.01	<0.01	0.14
20:2	0.17	0.15	0.08	0.14	0.02	0.03	0.21	0.07
22:0	0.08	0.08	0.07	0.09	0.03	0.85	0.66	0.81
20:3n6	0.08	0.06	0.05	0.07	0.02	0.64	0.91	0.32
20:4n6	0.15	0.18	0.14	0.16	0.01	0.27	0.24	0.71
23:0	0.20	0.24	0.07	0.15	0.1	0.30	0.55	0.84
22:2	0.14	0.12	0.08	0.12	0.02	0.14	0.78	0.23
24:0	0.12	0.12	0.08	0.08	0.01	0.01	0.11	0.96
PUFA <sup>3</sup>	7.11	7.54	8.19	10.15	0.71	0.03	0.13	0.31
MUFA	39.14	36.24	43.98	43.15	2.35	0.03	0.45	0.67
SFA	53.93	56.41	47.92	46.84	2.38	<0.01	0.78	0.47
ω 6	4.77	4.94	5.59	6.51	0.50	0.04	0.31	0.48
ω 3	1.08	1.06	0.98	1.08	0.17	0.82	0.81	0.70
PUFA/SFA	0.13	0.13	0.17	0.22	0.02	<0.01	0.22	0.27
ω 6/ω3	4.53	4.89	5.98	6.06	0.40	<0.01	0.59	0.74

<sup>1</sup>GF: Concentrate with ground flaxseeds. GFM: GF + monensin. GFP: GF pelleted. FMP: GF pelleted + monensin. <sup>2</sup>SEM: Standard error of the mean. P: Pelleting effect; M: Monensin effect. I: Interaction effect. <sup>3</sup>PUFA: Polyunsaturated FA. MUFA: Monounsaturated FA. SFA: Saturated FA.

**Table 4.** Intake and total apparent digestibility of nutrients by Holstein cows grazing on *Cynodon* pasture and supplemented with concentrate feed containing flaxseed.

	Non pelleted		Pelleted <sup>1</sup>		SEM	Probability <sup>2</sup>		
	GF	GFM	GFP	FMP		P	M	I
<i>Intake (Kg/d)</i>								
Dry matter	12.03	11.99	10.29	11.25	1.12	0.29	0.69	0.67
Crude protein	1.90	1.90	1.66	1.78	1.19	0.36	0.74	0.75
Ether extract	0.62	0.58	0.38	0.48	0.06	0.01	0.66	0.25
Neutral detergent fiber	4.78	5.10	4.10	4.41	0.49	0.19	0.53	0.10
Acid detergent fiber	2.26	2.39	1.92	2.11	0.23	0.21	0.51	0.91
<i>Digestibility (g/Kg)</i>								
Dry matter	622.6	635.6	666.0	644.9	1.54	0.12	0.80	0.30
Crude protein	677.6	699.2	719.8	695.8	1.78	0.30	0.95	0.23
Ether extract	837.9	865.6	847.3	868.7	2.16	0.78	0.29	0.89
Neutral detergent								
Fiber	560.8	588.9	594.3	545.7	2.69	0.86	0.71	0.19
<i>Acid detergent fiber</i>	532.1	569.3	555.3	514.7	2.96	0.61	0.96	0.22

<sup>1</sup>GF: Concentrate with ground flaxseeds. GFM: GF + monensin. GFP: GF pelleted. FMP: GF pelleted + monensin. <sup>2</sup>SEM: Standard error of the mean. P: Pelleting effect; M: Monensin effect. I: Interaction effect.

## Discussion

Monensin has a limited effect on DMI (Ipharraguerre and Clark 2003; Possatti *et al.*, 2015). Since it has the potential to increase the supply of gluconeogenic precursors, monensin administration might increase hepatic synthesis of glucose, thereby improving the energy balance in dairy cows (Ipharraguerre and Clark, 2003). Phipps *et al.* (2000) administered various doses of monensin (0, 150, 300, and 400 mg/d) and did not observe differences in DMI. Similar to the present study, previous works did not find effect of the thermal process in DMI (De Marchi *et al.*, 2013; 2015). Blood parameters are health indicators that can show the nutritional status and can be used to diagnose metabolic disorders. However, in the present study, no influence of the treatments on blood parameters was observed (data not shown), and all results remained within the recommended ranges.

The absence of effects on milk production and composition and nutrient digestibility could be attributed to the small amount of monensin ingested daily by the cows. Previous studies showed that milk production and composition, as well as DMI, were not affected in cows fed flaxseed and receiving 297 to 320 mg monensin/d (Petit *et al.*, 2009; Da Silva-Kazama *et al.*, 2011). Monensin acts directly on

gram-positive bacteria that are primarily acetate and methane producers, thus, reducing ruminal production of acetic acid (Bergen and Bates, 1984; Russell and Strobel, 1989). Since acetic acid is a precursor of short chain FA in the mammary gland (Chilliard *et al.*, 2000), monensin can reduce milk fat concentration up to 8%. Several reports on monensin effects on milk production and milk fat content range from decreases in milk production and milk fat content (Da Silva *et al.*, 2007) to increases in milk production and no effect on milk fat concentration (He *et al.*, 2012; De Marchi *et al.*, 2015). The monensin doses in those studies were different; therefore, we cannot claim that absence of effect in the present study was only a function of monensin amount. Indeed, the effects of monensin intake have shown to be less significant in cows fed roughage (Possatti *et al.*, 2015).

Despite the fact that monensin and pelleting did not have an effect on milk fat, the treatments, mainly pelleted diet, changed the FA profile of milk fat. The intake of food rich in SFA has been related to occurrence of coronary diseases and atherogenic effects due to increase of LDL cholesterol levels (Wang *et al.*, 2016). We observed satisfactory results as the pelleted diets decreased SFA C14, C18, C20, and C24 by approximately 21, 18.5, 14, and 33%, respectively. According to some studies, C14, C16 and C18 are

the major SFA present in the human diets. They have been indicated as the main dietary factors that raise LDL cholesterol and total blood cholesterol, and also are positively associated with the incidence of Type 2 diabetes (Noakes *et al.*, 1996; Calder, 2015; Mensink, 2016). Indeed, reduction of some SFAs can be related with PUFA content in the diet, since long FA chains, such as C18:3 present in flaxseed, are inhibitors of the mammary synthesis of short and medium FA chains (Chilliard *et al.*, 2000; Ward *et al.*, 2002). A reduction of C18:0 in milk content (approximately 18% in the present study) by pelleted diets was also observed by Dos Santos *et al.* (2011) and De Marchi *et al.* (2015), who reported a reduction of 17 and 15%, respectively. This FA is the final product of ruminal biohydrogenation, and its low concentration in milk could indicate that biohydrogenation was inhibited and maybe more intermediates of this process, such as CLA and C18:1 t11, were synthesized in the rumen and posteriorly transferred to the mammary gland. Based on the present results, we can suggest that pelleting was efficient in protecting PUFA from ruminal biohydrogenation.

A more pronounced effect of the treatments was observed on CLA concentration, once C18:2 c9 t11 was increased by approximately 47 and 70% by monensin and pelleted diets, respectively, with a greater amount of CLA when monensin was added in association with pelleting, similarly to the results observed by Gómez-Cortés *et al.* (2009) and Dos Santos *et al.* (2011).

Both pelleting and monensin act as inhibitors of PUFA biohydrogenation in the rumen (Fellner *et al.*, 1997; Neves *et al.*, 2009). Ionophores inhibit the growth of gram-positive bacteria, such as *Butyrivibrio fibrisolvens*, one of the main bacteria responsible for fatty acid hydrogenation, thus, PUFA conversion into SFA can be reduced. Furthermore, gram-positive bacteria produce hydrogen, and lower hydrogen availability may interfere the biohydrogenation process (Fellner *et al.*, 1997). Heat treatments of oil seeds alter the protein matrix that surrounds fat droplets. The protein matrix protects PUFA from biohydrogenation by a reduction in rumen degradation of fatty acids (Chouinard *et al.*, 1997). This way, intermediate products of biohydrogenation can be later incorporated into milk or further converted

into CLA by the delta-9-desaturase enzyme in the mammary gland.

The pelleted diet reduced milk SFA by 14% and increased PUFA, MUFA,  $\omega$ 6, PUFA/SFA, and  $\omega$ 6/ $\omega$ 3 by 25, 16, 25, 50, and 28%, respectively. Despite the small amount of monensin in the diets in the present study, it reflects the reality of many Brazilian farms where dairy cows are kept on pasture and receive concentrate feed. Our results suggest that, even at low amounts, monensin is able to improve milk FA quality of grazing cows, mainly increasing C18:2 c9 t11 (CLA) concentration. Pelleting and monensin do not alter milk production and composition. Pelleting, more than monensin, can improve milk fat quality once CLA, PUFA, and MUFA concentrations are increased in milk fat.

### Acknowledgment

The present study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil) in Brazil. Special thanks to Associação Paranaense dos Criadores de Bovinos da Raça Holandesa (APCBRH).

### Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

### References

- Bergen WG, Bates DB. Ionophores: their effect on production efficiency and mode of action. *J Anim Sci* 1984; 58:1465-1483.
- Calder PC. Functional roles of fatty acids and their effects on human health. *J Parenter Enteral Nutr* 2015; 39:18S-32S.
- Casali AO, Detmann E, Valadares Filho SC, Pereira JC, Henriques LT, De Freitas SG, Paulino MF. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos *in situ*. *Rev Bras Zootec* 2008; 37:335-342.
- Chilliard Y, Ferlay A, Mansbridge RM, Doreau M. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Ann Zootech* 2000; 49:181-205.
- Cochran RC, Adams DC, Wallace JD, Galyean ML. Predicting digestibility of different diets with internal markers: evaluation of four potential markers. *J Anim Sci* 1986; 63:1476-1483.



- Chouinard PY, Lévesque J, Girard V, Brisson GJ. Dietary soybeans extruded at different temperatures: Milk composition and in situ fatty acid reactions. *J Dairy Sci* 1997;80: 2913-2924.
- Corrêa LA, Santos PM. Manejo e utilização de plantas forrageiras dos gêneros *Panicum*, *Brachiaria* e *Cynodon*. São Carlos: Embrapa Pecuária Sudeste; 2003.
- Crumb DJ, Vatter, DA. Conjugated Linoleic Acid (CLA)-An Overview. *Intern J Appl Res Nat Produ* 2011; 4:12-18.
- Da Silva-Kazama DC, Cortes C, Kazama R, Benchaar C, Santos GT, Zeoula LM, Petit HV. Ruminal fermentation characteristics and fatty acid profile of ruminal fluid and milk of dairy cows fed flaxseed hulls supplemented with monensin. *J Dairy Res* 2011; 78:56-62.
- Da Silva DC, Santos GT, Branco AF, Damasceno JC, Kazama R, Matsushita M, Horst JA, Santos WBR, Petit HV. Production performance and Milk composition of dairy cows fed whole or ground flaxseed with or without monensin. *J Dairy Sci* 2007; 90:2928-2936.
- Da Silva-Kazama DC, Santos GT, Pinto PTM, Visentainer JV, Kazama R, Petit HV, De Marchi FE. Effect of storage on fatty acid profile of butter from cows fed whole or ground flaxseed with or without monensin. *Rev Bras Zootec* 2010; 39:2297-2303.
- De Marchi FE, Figueiroa FJF, Dos Santos GT, Dos Santos WBR, Da Silva-Kazama DC, Branco AF, Leite LC, Damasceno JC. Intake, digestibility and ruminal parameters of dairy cows fed pelleted diets and treated with lignosulfonate-containing sunflowerseeds. *Rev Bras Zootec* 2013; 42:656-663.
- De Marchi FE, Romero JV, Damasceno JC, Grande PA, Zeoula LM, Dos Santos GT. Pelleting in Associated with Sodium Monensin Increases the Conjugated Linoleic Acids Concentration in the Milk of Dairy Cows Fed Canola Seeds. *Asian-Aust J Anim Sci* 2015; 28:1095-1104.
- Detmann E, Paulino MF, Zervoudakis JT, Valadares Filho SC, Euclides RF, Lana RP, Queiroz, DS. Cromo e indicadores internos na determinação do consumo de novilhos mestiços, suplementados, a pasto. *Rev Bras Zootec* 2001; 30:1600-1609.
- Dos Santos WBR, Santos GT, Da Silva-Kazama DC, Cecato U, De Marchi FE, Visentainer JV, Petit HV. Production performance and milk composition of grazing dairy cows fed pelleted or non-pelleted concentrates treated with or without lignosulfonate and containing ground sunflower seeds. *Anim Feed Sci Technol* 2011; 169:167-175.
- Fellner V, Sauer FD, Kramer JKG. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. *J Dairy Sci* 1997; 80:921-928.
- Gómez-Cortés P, Bach A, Luna P, Juaréz M de la Fuente. Effects of extruded linseed supplementation on n-3 fatty acids and conjugated linoleic acid in milk and cheese from ewes. *J Dairy Sci* 2009; 92:4122-4134.
- He M, Perfield KL, Green HB, Armentano LE. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. *J Dairy Sci* 2012; 95:1447-1461.
- Ipharraguerre IR, Clark JH. Usefulness of ionophores for lactating dairy cows: a review. *Anim Feed Sci Technol* 2003; 106:39-57.
- ISO-International Organization for Standardization. Animal and vegetable fats and oils: preparation of methyl esters of fatty acids. Geneva, Switzerland, 1978, pp. 1-6.
- Mensink RP. Effects of saturated fatty acids on serum lipids and lipoproteins: a systematic review and regression analysis. World Health Organization, ISBN: 978 92 4 156534 9, 2016, 63p.
- NRC-National Research Council. Nutrient Requirements of Dairy Cattle. 7<sup>th</sup> Ed. Washington DC: National Academy Press; 2001.
- Neves CA, Dos Santos WBR, Santos GT, Da Silva DC, Jobim CC, Santos FS, Visentainer JV, Petit HV. Production performance and milk composition of dairy cows fed extruded canola seeds treated with or without lignosulfonate. *Anim Feed Sci Technol* 2009; 154:83-92.
- Petit HV, Côrtes C. Milk production and composition, milk fatty acid profile, and blood composition of dairy cows fed whole or ground flaxseed in the first half of lactation. *Anim Feed Sci Technol* 2010; 158:36-43.
- Paris W, Tonion R, Martinello C, Sartor LR, De Paula FLM, De Oliveira JG. Productivity and nutritional value of African Star managed with different leaf blade mass. *Acta Scientia. Anim Sci* 2016; 38:31-36.
- Petit HV, Côrtes C, Da Silva D, Kazama R, Gagnon N, Benchaar C, Dos Santos GT, Zeoula LM. The interaction of monensin and flaxseed hulls on ruminal and milk concentration of the mammalian lignan enterolactone in late-lactating dairy cows. *J Dairy Res* 2009; 76:475-482.
- Phipps RH, Wilkinson JID, Jonker LJ, Tarrant M, Jones AK, Hodge A. Effect of monensin on milk production of holstein-friesian dairy cows. *J Dairy Sci* 2000; 83:2789-2794.
- Possatti CD, Haddade IR, Kill JL, Haese D, Chambela N, Alberto S, Carla P, Rocha IA, Nascimento JVM do, Garcia WA. Monensina sódica sobre vacas em fase inicial de lactação: produção de leite e peso vivo. *Ciênc Rural* 2015; 45: 92-97.
- Pogliani FC, Birgel Junior E. Valores de referência do lipidograma de bovinos da raça holandesa, criados no Estado de São Paulo. *Braz J Vet Res Anim Sci* 2007; 44:373-383.
- Russell JB, Strobel HJ. Effect of ionophores on ruminal fermentation. *Appl Envir Microbiol* 1989; 55:1-6.
- Statistical Analysis System, Version 9.0 (TS Level 00M0) by SAS Institute Inc., Cary, NC, USA. 2002.
- Shingfield KJ, Bernard L, Leroux C, Chilliard Y. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. *Anim* 2010; 4:1140-1166.
- Sklan D, Ashkenazi R, Braun A, Devorin A, Taboru K. Fatty acids, calcium soaps of fatty acids and cottonseeds fed to high yielding cows. *J Dairy Sci* 1992; 75:2463- 2472.

Wang Q, Afshin A, Yakoob MY, Singh GM, Rehm CD, Khatibzadeh S, Micha R, Shi P, Mozaffarian D. Impact of Nonoptimal Intakes of Saturated, Polyunsaturated, and Trans Fat on Global Burdens of Coronary Heart Disease. *J Am Heart Assoc* 2016; 5:1-23.

Ward AT, Wittenberg KM, Przybylski R. Bovine milk fatty profile produced by feeding diets containing solin, flax and canola. *J Dairy Sci* 2002; 85:1191-1196.