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Ruminal fermentation and degradation, kinetic flow of digesta and milk fatty acid composition of cows fed sugarcane silage-based diets supplemented with whole cottonseed

Fermentação e degradação ruminal, cinética de fluxo da digesta e perfil de ácidos graxos do leite de vacas alimentadas com dietas à base de silagem de cana de açúcar suplementadas com caroço de algodão

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Highlights:
Nutrient digestibility is not affected by whole cottonseed in a sugarcane silage-based diet.
Kinetic flow of digesta is not affected by whole cottonseed in a sugarcane silage-based diet.
Oleic acid in milk fat is improved by whole cottonseed in a sugarcane silage-based diet.
Hypercholesterolemic acids in milk fat are decreased by whole cottonseed.
Rumenic acid in milk fat is not affected by whole cottonseed in a sugarcane silage-based diet.

Abstract

The aim of this study was to evaluate the ruminal fermentation and degradation parameters, the kinetic flow of digesta in the gastrointestinal tract, and the milk fatty acid (FA) profile in Holstein x Gyr cows fed 59% sugarcane silage-based diets with 0% (control), 5%, 10% and 15% whole cottonseed (WCS) on a dry matter (DM) basis. Four rumen-cannulated cows with an average milk yield of 14.4±3.3 kg day^{-1} and 85±25 days in milk were allocated in a 4 x 4 Latin square design. There was no effect of dietary WCS levels on the intake of organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and nonfibrous carbohydrates, but there was a linear increase in the intake of ether extract (EE). There was no treatment effect on the apparent digestibility of DM, OM, CP, EE and NDF or on the ruminal degradability parameters for DM and NDF of sugarcane silage. Also, no treatment effects were observed on rumen pH, ruminal and postruminal particulate passage rates or on the rate of passage of the fluids in the rumen. WCS promoted linear reductions in the milk fat contents of lauric, myristic and palmitic acids and linear increases in the milk fat contents of stearic, oleic, *trans*-10 C18:1 and elaidic

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acids. The milk fat contents of vaccenic, rumenic, linoleic and α-linolenic acids were unaffected by WCS supplementation. The inclusion of up to 15% WCS in sugarcane silage-based diets did not alter the digestibility of nutrients, the rumen degradability of the fiber, or the kinetic flow of digesta in the gastrointestinal tract of Holstein x Gyr cows but improved the nutritional quality of milk fat through an increase in the content of oleic acid, which is beneficial to human health, and decreased levels of hypercholesterolemic lauric, myristic and palmitic acids.
Key words: Conjugated linoleic acid. *Saccharum officinarum*. Rate of passage. Rumen.

Resumo

O objetivo deste estudo foi avaliar os parâmetros de fermentação e degradação ruminal, a cinética de fluxo da digesta no trato gastrintestinal, e o perfil de ácidos graxos do leite de vacas Holandês x Gir alimentadas com dietas à base de 59% de silagem de cana de açúcar com 0% (controle), 5%, 10% e 15% de caroço de algodão na base de matéria seca (MS). Quatro vacas canuladas no rúmen, com produção de leite de 14,4±3,3 kg dia^{-1} *e* 85±25 dias em lactação foram alocadas em delineamento quadrado latino 4 x 4. Não houve efeito da inclusão do caroço de algodão sobre os consumos de matéria orgânica (MO), proteína bruta (PB), fibra em detergente neutro (FDN) e de carboidratos não fibrosos, mas houve aumento linear no consumo de extrato etéreo (EE). Não houve efeito dos tratamentos sobre a digestibilidade da MS, MO, PB, EE e FDN nem sobre os parâmetros de degradabilidade ruminal da MS e FDN da silagem de cana de açúcar. Não houve efeito dos tratamentos sobre o pH ruminal, taxas de passagem de fluidos e partículas no rúmen, nem sobre a taxa de passagem pós-ruminal de partículas. Houve reduções lineares nos teores dos ácidos láurico, mirístico e palmítico, e aumentos lineares nos teores dos ácidos esteárico, oleico, C18:1 *trans*-10 e elaídico no leite. A inclusão de caroço de algodão não alterou os teores dos ácidos vacênico, rumênico, linoleico e α-linolênico no leite. A inclusão de até 15% de caroço de algodão em dietas à base de silagem de cana de açúcar não alterou a digestibilidade dos nutrientes, a degradabilidade ruminal da fibra, nem a cinética de fluxo da digesta no trato gastrintestinal de vacas Holandês x Gir, mas melhorou a qualidade nutricional da gordura do leite, com aumento no teor de ácido oleico, benéfico para a saúde humana, e redução nos teores dos ácidos hipercolesterolêmicos láurico, mirístico e palmítico.
Palavras-chave: Ácido linoleico conjugado. Rúmen. *Saccharum officinarum*. Taxa de passagem.

Introduction

Ruminant milk fat contains a number of biologically active fatty acids (FA) with potential positive effects on human health. The main isomer of CLA (conjugated linoleic acid) in ruminant milk fat is rumenic acid (*cis*-9, *trans*-11 CLA), to which anticarcinogenic, antidiabetogenic (type 2 diabetes), antiatherogenic and immunomodulatory properties have been attributed (Yang et al., 2015). Another important biologically active FA in ruminant milk fat is vaccenic acid (*trans*-11 C18:1), which accounts for 70-95% of the total amount of rumenic acid secreted in bovine milk (Kliem & Shingfield, 2016). Oleic acid (*cis*-9 C18:1), which is associated with a reduction in plasma LDL-cholesterol levels (Food and Agriculture Organization of the United Nations [FAO], 2010), is the second most abundant FA in bovine milk fat under most dietary conditions (Lopes, Silva, Almeida, & Gama, 2015). Another health-enhancing FA naturally present in bovine milk fat is α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3), which is essential for human metabolism and a precursor of other ω-3 FAs, which are considered to possess cardioprotective and anti-inflammatory properties (FAO, 2010).

Sugarcane (*Saccharum officinarum* L.) is a source of roughage traditionally used in dairy farms in Brazil during the dry season when the growth of other tropical grasses is drastically reduced. Due to agronomic advantages, sugarcane silage

has often been used by dairy farmers in Brazil as a feasible strategy to feed cows during the dry season (Marcondes, Gionbelli, & Andrade, 2011). Urea is a recommended additive in sugarcane silage, promoting increases in dry matter (DM), crude protein (CP) (Sousa et al., 2008; Marcondes et al., 2011) and organic acid content of the silage, as well as reducing ethanol production during the fermentation (Sousa et al., 2008). Diets based on sugarcane silage have been shown to allow the production of up to 20 kg day^{-1} of milk as long as supplemental energy and protein sources are provided (Marcondes et al., 2011).

Low contents of rumenic acid have been reported in milk fat from cows fed diets based on fresh sugarcane (Meneses et al., 2015; Pimentel et al., 2016; Souza et al., 2019) or sugarcane silage (Martins et al., 2016) without lipid supplementation. However, a marked increase (~388-395%) in milk fat rumenic acid content was found when cows were fed fresh sugarcane-based diets supplemented with ingredients rich in oleic and linoleic (*cis*-9, *cis*-12 C18:2) acids (Meneses et al., 2015; Souza et al., 2019).

Whole cottonseed (WCS) is a concentrate feed with desirable nutritional characteristics for inclusion in diets of lactating cows, and inclusion levels of up to 15% of diet DM have been recommended (Arieli, 1998). In addition to the effectiveness of the fibrous fraction of WCS (Arieli, 1998) and the high contents of CP, ether extract (EE) and energy (Coppock, Lanham, & Horner, 1987; Arieli, 1998), the oil present in WCS is rich in unsaturated FAs, notably oleic and linoleic acids (Chen, Ji, & Li, 2008), which can improve the FA composition and the nutritional quality of milk fat.

To the best of our knowledge, no studies have been conducted to evaluate the effects of WCS supplementation on the milk FA profile of dairy cows fed diets based on fresh sugarcane or on sugarcane silage. In corn silage-based diets, supplementation with 10-20% WCS (on a DM basis) promoted an increase in the nutritional quality of milk fat with a reduction in the contents of myristic (C14:0) and palmitic (C16:0) acids and an increase in oleic acid content (Chen et al., 2008; Dayani, Ghorbani, & Esmailizadeh, 2011). However, no effect of WCS was observed in the milk fat contents of rumenic, α-linolenic (Chen et al., 2008; Dayani et al., 2011) and vaccenic acids (Dayani et al., 2011).

The aim of this study was to evaluate the ruminal fermentation and degradation parameters, the kinetic flow of digesta in the gastrointestinal tract, and the milk fatty acid profile in Holstein x Gyr cows fed sugarcane silage-based diets supplemented with increasing levels of whole cottonseed.

Materials and Methods

The study was carried out at Embrapa Dairy Cattle (Coronel Pacheco, MG, Brazil) from October 2009 to January 2010, and the experimental procedures used were approved by the Ethics Committee in Animal Experimentation (CETEA/UFMG Protocol #163/2009). Four multiparous (3-4 lactations) 3/4 to 15/16 Holstein x Gyr cows (438±35 kg) with 85±25 days in milk and producing 14.4±3.3 kg day^{-1} of milk at the beginning of the study were used. A 4 x 4 Latin square (LS) experimental design with 18 d-periods was used, with the first 10 days being employed for diet adaptation and the remaining eight days being employed for sampling and data recording.

The experimental diets were composed of sugarcane silage (59% of diet DM) containing 0% (control), 5%, 10% or 15% whole cottonseed (WCS) with lint on a DM basis (Table 1). The sugarcane was ensiled with 1% urea (as-fed basis). The cows were allocated in a free stall and were provided with water and a mineral mixture. The free stall was equipped with electronic troughs (American Calan Inc., Northwood, NH, USA), which allowed individual control of food intake. The diets were provided to cows as a TMR using a mixer/dispenser vehicle (Data Ranger; American Calan Inc.). Diets

were supplied in amounts to allow for 10% orts into two meals, with 60% being provided at 07h00 and the remainder at 16h00.

Individual fecal DM production was estimated using titanium dioxide (TiO_2) as an external marker at a rate of 10 g cow^{-1} day^{-1}, administered orally and wrapped in paper for 11 days in two doses of 5 g each immediately after each milking. During the last five days of TiO_2 administration, fecal samples were individually collected by rectal grabbing twice a day after each milking and stored (-10°C). At the end of the experiment, the fecal samples were thawed, predried (55°C, 72 h), milled (5 mm), and then pooled by cow*LS period based on predried DM. Afterwards, the composite fecal samples were ground again to pass through a 1-mm screen and stored for later analysis of TiO_2 content according to Myers, Ludden, Nayigihugu and Hess (2004). Fecal DM production was determined by the ratio between the amount of the marker administered to the cow (g day^{-1}) and its concentration in fecal DM, expressed as g kg^{-1} (Lopes, 2007), and the apparent digestibility of the nutrients was estimated according to Wiseman (2018).

Table 1
Ingredients and chemical composition of the experimental rations

Item	Whole cottonseed (WCS) in the diet (%DM)			
	0	5	10	15
Ingredient, %DM				
Sugarcane silage[a]	59.1	59.2	59.0	59.2
Ground corn	23.0	21.7	21.0	19.7
Cottonseed meal	16.4	12.6	8.5	4.6
WCS	0.0	5.0	10.0	15.0
Mineral/vitamin supplement	1.5	1.5	1.5	1.5

Chemical composition, %DM						
Item	Sugarcane silage[a]	WCS	WCS in the diet (%DM)			
			0	5	10	15
DM, % of as fed	21.2	87.9	32.4	32.9	31.4	32.3
Crude protein	11.1	23.4	15.9	16.5	17.2	16.8
Ether extract	0.5	18.8	1.8	2.5	2.7	4.8
Neutral detergent fiber	65.4	49.0	49.9	48.0	48.4	44.7
Non fibrous carbohydrates	15.4	5.5	24.0	25.0	23.2	25.6

[a]Ensiled with 1% urea (as-fed basis).

From the 14th to the 18th day of each LS period, the intake of each cow was measured as the difference between the daily quantity of feed that was supplied and its respective ort. Samples of the silage sugarcane, WCS and the four concentrates were collected and stored (-10°C). Samples of the individual orts were also collected and transformed into composites by cow*LS period and stored (-10°C). At the end of the experiment, these samples were thawed, predried (55°C, 72 h), milled (1 mm) and analyzed in the Laboratory of Food Analysis of Embrapa Dairy Cattle for DM (at 105°C), CP, EE, mineral matter and neutral detergent fiber (NDF) content (Detmann et al., 2012).

On the 15th day of each LS period, individual milk samples (2/3 at morning milking + 1/3 at afternoon

milking) were collected and stored (-20°C) in flasks with no preservative to determine the FA profile using a GC model 6890N (Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a 100 m x 0.25 mm x 0.2 μm column (CP-SIL 88; Varian, Inc., Mississauga, ON, USA) and equipped with a flame ionization detector (FID). The analysis was performed at the Laboratory of Chromatography of Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) using the analytical procedures described in detail elsewhere (Mourthé et al., 2019).

The nutritional quality of milk fat was evaluated by the atherogenicity (AI) and thrombogenicity (TI) indexes and by the relationships between omega 6 and omega 3 FAs (ω-6/ω-3 FA ratio) and between hypo- and hypercholesterolemic FAs (h/H FA ratio) according to the equations presented by Mourthé et al. (2019).

The ruminal parameters were evaluated on the 11th and 12th days of each LS period. Samples of ruminal fluid were collected before (time 0) and 2, 4, 6, 8, 10, 12, 16 hours (11th day) and 24 hours (12th day) after the provision of the morning rations. The samples were filtered using gauze, and the pH was measured using a digital potentiometer. Two 10 mL aliquots were added to flasks with 0.4 mL of sulfuric acid (50% v/v, subsample 1) or 2 mL of metaphosphoric acid (25% v/v, subsample 2) and frozen. Subsample 1 was analyzed for the ammonia N content (N-NH_3) according to the INCT-CA N-007/1 method (Detmann et al., 2012). Subsample 2 was centrifuged (17,000 x *g* for 10 min) and analyzed for the molar concentration (mMol 100 mL^{-1}) of the volatile fatty acids (VFA) using GC model 7820A (Agilent Technologies, Inc.) with a FID and Nukol capillary column (30 m x 22 mm x 0.25 μm) connected to free FA (SUPELCO, Bellefonte, PA, USA).

For the study of ruminal degradability (Nocek, 1988), a predried (55°C, 72 h) and milled (5 mm) sample of sugarcane silage was used, which was conditioned in nylon bags (10 x 20 cm, 50 μ porosity, 10-20 mg of sample per cm^2) incubated in the rumen of each cow in each LS period. The nylon bags for time 0 were washed and frozen, and the remaining bags were incubated in the rumen and removed 6, 24 and 96 hours later and frozen. Subsequently, they were washed, predried (55°C, 72 h), and weighed, and the residues were analyzed for DM and NDF contents (Detmann et al., 2012). The ruminal degradability parameters were estimated by adjusting the degradation data as a function of time, to a nonlinear model (Tomich & Sampaio, 2004) using the NLIN procedure of SAS version 9.1. Curves were generated for each cow in each LS period. The effective degradabilities were calculated (Ørskov & McDonald, 1979) based on the estimated particulate passage rates in the rumen.

In the study of the kinetics of particulate passage, a sugarcane silage sample was subjected to hot extraction with a neutral detergent, obtaining a fibrous fraction, which was complexed with sodium dichromate according to Udén, Colucci and Van Soest (1980). For each LS period, 100 g of chromium-mordanted NDF (1.9-2.2% Cr, DM basis) was intra-ruminally administered in a single dose to each cow, and fecal samples were collected from the rectum at 6, 12, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 84, 92, 96, 104, 120, 128 and 144 hours after dosing with the mordant. Fecal samples were predried (55°C; 72 h), milled (1 mm) and analyzed for Cr content by atomic absorption spectrophotometry INCT-CA M-005/1 method (Detmann et al., 2012). For the study of the kinetics of the fluids in the rumen, in each LS period, before the provision of the morning rations, 5 g of Co-EDTA (17.2% Co, DM basis), obtained according to Udén et al. (1980), was intra-ruminally administered to the cows, after being diluted in 200 mL of distilled water. From the ruminal fluid samples collected for the study of the fermentation parameters, 5 mL was further pipetted, transferred to tubes, stored (-10°C), and then analyzed for Co content by atomic absorption spectrophotometry. The parameters of the kinetics of particulate

and fluids passage were estimated for each cow using the NLIN procedure of SAS. The fecal Cr contents as a function of time were adjusted to a two-compartment biexponential time-independent model (Grovum & Williams, 1973) and to a multi-compartmental model (Dhanoa, Siddons, France, & Gale, 1985), while the Co content in ruminal fluid was adjusted to an unicompartmental exponential model (Valadares & Pina, 2011).

The results were analyzed by mixed models using the MIXED procedure of SAS. The ruminal fermentation parameters were analyzed as repeated measures in time and were considered to be fixed effects: treatment (% WCS in diet), sampling time and interaction between these factors, and random effects: cow, LS period and the interaction cow*LS period*treatment. Ten matrices of covariance were evaluated based on the Akaike information criterion. The other variables were analyzed considering treatment as a fixed effect and cow and LS period as random effects. The linear and quadratic effects were analyzed using orthogonal contrasts. The results are reported as least squares means. Significant differences were declared at $P \leq 0.05$. Pearson correlations between specific variables were obtained by the CORR procedure of SAS.

Results

There was no effect ($P>0.05$) of the inclusion of WCS on the intake of organic matter (OM), CP, NDF and nonfibrous carbohydrate (NFC), but there was a linear increase ($P<0.01$) in the intake of EE. There was no effect ($P>0.05$) of WCS on the apparent digestibility of DM, OM, CP, EE and NDF (Table 2) or on the ruminal degradability parameters for DM and NDF of the sugarcane silage (Table 3).

Table 2
Intake and apparent digestibility of nutrients in Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Item	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
Nutrient intake (kg day^{-1})							
Organic matter (OM)	9.46	9.74	10.24	8.26	1.0654	0.4469	0.2295
Crude protein (CP)	1.61	1.74	1.94	1.52	0.1952	0.9152	0.1357
Ether extract (EE)	0.19	0.26	0.29	0.43	0.0599	0.0261	0.5901
Neutral detergent fiber (NDF)	5.00	5.07	5.29	4.16	0.7263	0.0864	0.0541
Non fibrous carbohydrates	2.66	2.66	2.73	2.59	0.4262	0.9473	0.8633
Nutrient intake (kg 100 kg^{-1} of body weight)							
OM	1.87	1.95	2.10	1.62	0.1844	0.3446	0.0782
NDF	0.99	1.01	1.08	0.87	0.1288	0.3330	0.1168
Apparent nutrient digestibility (%)							
Dry matter	59.3	64.0	63.3	61.8	3.5290	0.6412	0.3448
OM	61.1	65.8	64.6	63.4	3.3446	0.6836	0.3581
CP	66.1	68.9	71.6	70.8	2.7820	0.0867	0.3617
EE	78.9	79.2	83.1	90.7	6.4279	0.1417	0.5062
NDF	43.0	46.6	47.3	35.7	7.6310	0.3042	0.1226

Table 3
Parameters of the dry matter (DM) and neutral detergent fiber (NDF) degradability of sugarcane silage in cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Parameter	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
Ruminal DM degradability							
Potential degradability (%)	55.6	55.9	56.3	58.4	4.2497	0.5016	0.7452
Degradation rate (% hour^{-1})	2.30	3.66	3.24	2.21	0.7160	0.8146	0.0983
Effective degradability (%)[a]	41.8	42.2	42.3	42.1	1.9272	0.8976	0.8558
Effective degradability (%)[b]	42.4	42.7	42.6	42.4	1.7161	0.9938	0.8864
Ruminal NDF degradability							
Potential degradability (%)	45.8	48.5	46.7	43.2	4.9056	0.4641	0.2170
Degradation rate (% hour^{-1})	3.20	3.09	3.80	2.77	0.6759	0.8313	0.4078
Effective degradability (%)[a]	24.6	26.2	26.0	26.7	1.9608	0.4424	0.7744
Effective degradability (%)[b]	25.8	27.0	26.4	27.2	1.9525	0.6212	0.8691

[a, b]ED = effective degradability, calculated considering the passage rate of solids in the rumen estimated by the Grovum and Williams (1973) and Dhanoa et al. (1985) model, respectively (Table 5).

There was an effect (P<0.01) of the interaction treatment*sampling time on all rumen fermentation parameters, except for pH (Table 4). No effect (P>0.05) of the treatments on the rumen pH was observed, but there was an effect of sampling time (P=0.0001) (Table 4), for which the minimum value was 6.06, estimated to occur at 8.5 h after the provision of the morning ration (Figure 1). There was no effect (P>0.05) of WCS on the rate of passage of fluids in the rumen or on the rates of particulate passages in the gastrointestinal tract of the cows (Table 5).

Table 4
Ruminal fermentation parameters in Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Item	WCS in the diet (%DM)				SEM	P-value				
	0	5	10	15		Linear	Quad	Diet	Time	D x T[a]
pH	6.35	6.31	6.26	6.22	0.1018	0.3574	0.9536	0.8124	0.0001	0.0746
N-NH_3 (mg dL^{-1})	21.76	18.50	23.58	16.99	1.9855	0.3725	0.4433	0.1096	0.0034	0.0007
Acetate (A)[b]	34.22	37.66	32.67	28.88	1.6386	<0.0001	0.0002	0.0001	0.1312	<0.0001
Propionate (P)[b]	14.21	15.52	14.48	11.27	0.7348	0.0080	0.0058	0.0038	0.0001	0.0001
Butyrate[b]	5.66	7.08	5.82	4.87	0.6549	0.2070	0.0693	0.1048	<0.0001	<0.0001
VFA[c]	54.69	60.26	52.97	45.02	2.7706	<0.0001	<0.0001	<0.0001	0.0045	0.0018
A: P ratio	2.43	2.46	2.30	2.78	0.1866	0.2966	0.1850	0.1613	0.0001	0.0048

[a]Interaction between treatment (% WCS in the diet) and sampling time; [b]mMol 100 mL^{-1}; [c]VFA (volatile fatty acids; mMol 100 mL^{-1}) = Σ ruminal concentrations of acetate + propionate + butyrate.

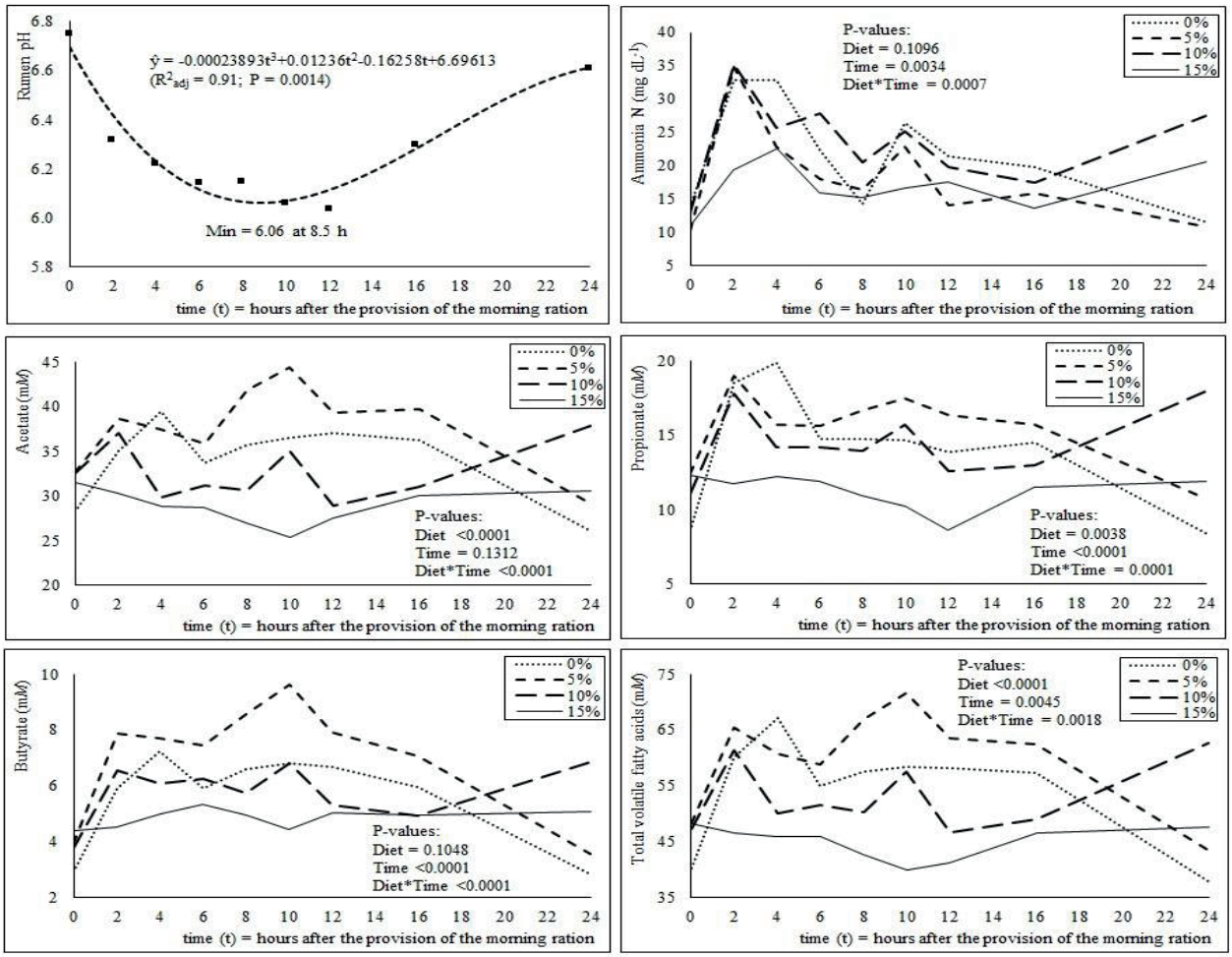

Figure 1. Effect of sampling time (t, hour) on fermentation parameters in the rumen of Holstein x Gyr cows fed sugarcane silage-based diets containing 0%, 5%, 10% or 15% whole cottonseed.

Table 5
Rates of passage of fluids and solids of digesta in Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Rate of passage (RP)	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
RP_{fluids} (% hour^{-1})[a]	9.47	7.85	8.63	8.92	0.6596	0.7086	0.1000
$k_{1\text{-}Grovum}$ (% hour^{-1})[b]	3.72	3.63	3.48	3.57	0.2115	0.5213	0.6799
$k_{2\text{-}Grovum}$ (% hour^{-1})[b]	9.11	9.24	9.19	7.13	1.5972	0.1896	0.2731
$k_{1\text{-}Dhanoa}$ (% hour^{-1})[c]	3.31	3.41	3.31	3.36	0.2672	0.9524	0.9256
$k_{2\text{-}Dhanoa}$ (% hour^{-1})[c]	12.66	9.15	9.75	8.51	1.6176	0.0652	0.4925

[a]RP of fluids in the rumen; [b]$k_{1\text{-}Grovum}$ and $k_{2\text{-}Grovum}$ = respectively, solids RP in the rumen and in the caecum/proximal colon estimated by the Grovum and Williams (1973) model; [c]$k_{1\text{-}Dhanoa}$ and $k_{2\text{-}Dhanoa}$ = respectively, solids RP in the rumen and in the caecum, estimated by the Dhanoa et al. (1985) model.

WCS promoted linear reductions (P<0.05) in the milk fat contents of lauric (C12:0), myristic and palmitic acid and linear increases (P<0.05) in the milk fat contents of stearic (C18:0) (Table 6), oleic, *trans*-10 C18:1 and elaidic acid (*trans*-9 C18:1) (Table 7). The inclusion of WCS did not alter (P>0.05) the milk fat contents of vaccenic (Table 7), rumenic, linoleic and α-linolenic acid but promoted a quadratic effect (P=0.0383) on the milk fat content of *trans*-10, *cis*-12 CLA (Table 8) with a maximum value of 0.0114 g 100 g^{-1} FA, estimated for the ration with 7.9% WCS. The milk fat content of the major odd-chain and branched-chain FAs (OBCFA) were linearly reduced (P<0.05) with the inclusion of WCS (Table 6).

Table 6
Linear even-chain saturated fatty acids and odd- and branched-chain fatty acids (OBCFA) in milk fat of cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Fatty acid – FA (g 100 g^{-1} FA)	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
C4:0 + C6:0 + C8:0 + C10:0	10.61	10.37	8.73	7.97	0.6918	0.0030	0.5840
C12:0	4.12	3.14	2.54	2.09	0.2285	0.0001	0.1585
C14:0	12.91	10.94	9.76	7.88	0.6275	0.0002	0.9246
C16:0	30.54	26.71	27.92	26.23	1.2949	0.0106	0.2292
C12:0 + C14:0 + C16:0	47.57	40.64	40.23	36.20	1.8827	0.0002	0.2586
C18:0	8.63	13.36	14.96	16.28	1.3815	0.0008	0.0946
C18:0 + C20:0 + C22:0 + C24:0	8.89	13.67	15.24	16.54	1.4104	0.0009	0.0946
iso C15:0	0.30	0.28	0.24	0.24	0.0199	0.0114	0.5809
anteiso C15:0	0.75	0.72	0.64	0.55	0.0635	0.0058	0.5104
C15:0	1.49	1.22	1.03	0.83	0.1091	0.0006	0.6044
C17:0	0.56	0.51	0.47	0.45	0.0270	0.0036	0.5303
cis-9 C17:1	0.24	0.18	0.18	0.20	0.0250	0.0969	0.0201
Σ OBCFA	3.92	3.44	3.01	2.68	0.2264	0.0003	0.6276

Table 7
***cis*- and *trans*-Monounsaturated fatty acids in milk fat of Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)**

Fatty acid – FA (g 100 g^{-1} FA)	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
cis-9 C14:1	1.39	1.02	0.84	0.62	0.1073	<0.0001	0.2225
cis-9 C16:1	2.07	1.69	1.57	1.55	0.1791	0.0004	0.0135
cis-9 C18:1	16.50	19.66	21.72	25.49	1.1018	<0.0001	0.5984
cis-11 C18:1	0.52	0.48	0.47	0.54	0.0323	0.6086	0.0574
cis-12 C18:1	0.11	0.16	0.18	0.20	0.0088	<0.0001	0.1021
cis-13 C18:1	0.054	0.054	0.057	0.071	0.0049	0.0184	0.1133
trans-9 C16:1	0.31	0.28	0.27	0.31	0.0168	0.8106	0.0392
trans-4 C18:1	0.011	0.022	0.022	0.021	0.0025	0.0009	0.0025

continue

continuation

trans-5 C18:1	0.011	0.016	0.016	0.016	0.0015	0.0061	0.0160
trans 6-8 C18:1	0.16	0.23	0.23	0.26	0.0141	0.0001	0.0147
trans-9 C18:1	0.15	0.17	0.19	0.20	0.0117	0.0040	0.2547
trans-10 C18:1	0.23	0.29	0.29	0.30	0.0317	0.0365	0.1887
trans-11 C18:1	0.76	0.98	0.82	0.83	0.0810	0.8757	0.1540
trans-12 C18:1	0.13	0.21	0.25	0.30	0.0209	<0.0001	0.2607
trans 13-14 C18:1	0.19	0.26	0.27	0.21	0.0333	0.2935	0.0080
trans-16 C18:1	0.12	021	0.27	0.32	0.0240	<0.0001	0.1829
Σ *trans*-C18:1	1.76	2.39	2.36	2.46	0.1793	0.0065	0.0633

Table 8
Conjugated isomers of linoleic acid (CLA), omega 6 (ω-6) and omega 3 (ω-3) fatty acids in milk fat of cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Fatty acid – FA (g 100 g^{-1} FA)	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
cis-9, *trans*-11 CLA	0.41	0.42	0.37	0.37	0.0400	0.2153	0.8667
trans-9, *cis*-11 CLA	0.012	0.012	0.012	0.012	0.0009	0.9325	0.7305
trans-10, *cis*-12 CLA	0.009	0.011	0.011	0.009	0.0012	0.6105	0.0383
cis-9, *cis*-12 C18:2 (ω-6)	1.66	1.62	1.43	1.55	0.1046	0.1590	0.3255
cis-6, *cis*-9, *cis*-12 γ-C18:3 (ω-6)	0.025	0.024	0.024	0.025	0.0027	0.8678	0.4664
cis-9, *cis*-12, *cis*-15 C18:3 (ω-3)	0.086	0.068	0.059	0.070	0.0082	0.0792	0.0643
cis-11, *cis*-14 C20:2 (ω-6)	0.028	0.028	0.024	0.021	0.0020	0.0084	0.5090
cis-8, *cis*-11, *cis*-14 C20:3 (ω-6)	0.084	0.083	0.076	0.073	0.0081	0.0795	0.8541
cis-5, *cis*-8, *cis*-11, *cis*-14 C20:4 (ω-6)	0.20	0.16	0.15	0.14	0.0138	0.0056	0.1607
C20:5 EPA (ω-3)	0.015	0.011	0.011	0.011	0.0019	0.1318	0.3996
C22:5 DPA (ω-3)	0.052	0.050	0.047	0.048	0.0047	0.2640	0.6489
Σ ω-3 *cis* fatty acids	0.15	0.13	0.12	0.13	0.0115	0.0815	0.0964
Σ ω-6 *cis* fatty acids	2.00	1.91	1.71	1.81	0.1196	0.0967	0.3124

EPA = *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5; DPA = *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:5.

WCS promoted a linear reduction in SCD activity for the pair of FAs *cis*-9 C14:1/myristic (P=0.010) and a quadratic effect (P<0.05) on the pairs of FAs *cis*-9 C16:1/palmitic and oleic/stearic, but there was no effect (P=0.0538) on the pair of FAs rumenic/vaccenic (Table 9).

Table 9
Indices of stearoyl-CoA desaturase (SCD) enzyme activity in Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Indice	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
cis-9 C14:1/(*cis*-9 C14:1 + C14:0)	0.098	0.083	0.079	0.073	0.0077	0.0010	0.1693
cis-9 C16:1/(*cis*-9 C16:1 + C16:0)	0.064	0.059	0.053	0.056	0.0058	0.0022	0.0296
cis-9 C18:1/(*cis*-9 C18:1 + C18:0)	0.657	0.593	0.597	0.610	0.0256	0.0616	0.0348
Rumenic/(rumenic + *trans*-11 C18:1)	0.346	0.304	0.313	0.310	0.0201	0.0538	0.0979

WCS promoted linear reductions in AI and TI (P<0.0001) and a linear increase (P=0.0006) in the h/H FA ratio, but there was no effect (P>0.05) on the ω-6/ω-3 FA ratio (Table 10).

Table 10
Indices of the nutritional quality of milk fat from Holstein x Gyr cows fed sugarcane silage-based diets containing increasing amounts of whole cottonseed (WCS)

Indice	WCS in the diet (%DM)				SEM	P-value	
	0	5	10	15		Linear	Quadratic
Atherogenicity indice	4.69	3.38	3.02	2.20	0.3222	<0.0001	0.2271
Thrombogenicity indice	5.38	4.58	4.40	3.56	0.3123	<0.0001	0.9871
Hypo/hypercolesterolemic fatty acids ratio	0.36	0.49	0.58	0.71	0.0505	0.0006	0.7680
ω-6/ω-3 fatty acids ratio	13.1	15.1	14.8	14.1	0.9604	0.4156	0.1215

Discussion

The similarity in the OM, CP and NFC intakes (P>0.05) can be explained by the similar concentrations of these nutrients in the diets (Table 1) and by the similar DM intakes among treatments, as presented in a companion paper by Costa et al. (2011). These authors reported that the inclusion of WCS in the diet did not affect (P>0.05) the DM intake (expressed in kg cow^{-1} day^{-1} or in % of body weight). The NDF content analyzed in the 15% WCS diet was slightly lower than that of the other diets (Table 1), and in relation to the control treatment, the NDF intake of the cows that received 15% WCS decreased by ~17% (expressed in kg cow^{-1} day^{-1}) and ~12% (expressed in % of body weight). However, despite this finding, there was no effect (P>0.05) of WCS inclusion in the diet on NDF intake (Table 2). As expected, the substitution of cottonseed meal and ground corn by WCS rich in fat (Table 1) resulted in a linear increase (P=0.0261) in EE intake (Table 2). In the present study, the EE contents of the diets (Table 1) did not reach the maximum value of 6-7% EE (DM basis) recommended by the National Research Council [NRC] (2001) to avoid a decrease in digestion of the fibrous fraction of the diet and therefore in the DM intake. However, in formulating a diet for lactating cows, attention should be paid not only to the EE content of the diet but also to the rate at which unsaturated FAs are released from feeds to ruminal fluid. This parameter is the main aspect that will determine how ruminal fermentation is affected (NRC, 2001), since the fibrolytic bacteria, which are the main microorganisms responsible

for the biohydrogenation (BH) processes of FAs in the rumen, are highly vulnerable to the bacteriostatic effects of polyunsaturated FAs. According to NRC (2001), feeding polyunsaturated oils as part of a whole-oilseed diet has minimal effects on fermentation, probably because the oil is released slowly from the seed to ruminal fluid. This phenomenon occurs with the WCS, where the polyunsaturated FAs are protected inside the seed, requiring greater effort of rumination and mastication for its release (Nogueira, Perna, Pereira, & Rodrigues, 2019), providing small amounts of lipids in the ruminal environment, which may result in rapid BH, avoiding unsaturated FA accumulation and impairment of the ruminal degradation of the fiber. Moreover, the amount will not be enough to adhere to feed particles, causing physical impediment to microorganisms and microbial enzymes (Bettero et al., 2013). Thus, in the present study, the lack of effect of the inclusion of WCS on the parameters of ruminal DM and NDF degradability (Table 3) and on apparent digestibility of the nutrients (Table 2) could be linked to slow release of lipids in the rumen by WCS, not exceeding the hydrogenating capacity and preventing potential deleterious effects on the resident microbiota, fiber digestion and DM intake (Almeida et al., 2016).

The results obtained (Tables 2 and 3) corroborate those presented by Sousa et al. (2009) and Bettero et al. (2013). In an *in vitro* assay, the inclusion of 10% WCS in a diet based on 55% corn silage did not change the potential degradability, degradation rate or effective degradability of NDF (P>0.05) (Bettero et al., 2013). Sousa et al. (2009) reported that the inclusions of 7% or 14% WCS in chopped sugarcane-based diets did not alter the digestibility of DM, OM, CP, NDF and NFC or the CP and NDF intakes (kg day^{-1} cow^{-1}) by 7/8 Holstein x Gyr cows. As observed in the present study, in other studies, increased EE intake (kg cow^{-1} day^{-1}) was also reported in response to inclusion of WCS in the diet (Sousa et al., 2009; Dayani et al., 2011). However, in the present study, despite the ~15% increase in the EE digestibility observed in the treatment with 15% WCS in relation to the control, there was no effect of WCS on EE digestibility (P>0.05) (Table 2). In a chopped sugarcane-based diet, Sousa et al. (2009) reported that in relation to the control treatment, the inclusion of 14% WCS promoted an increase of ~9% in EE digestibility (P<0.05).

The ruminal environment of the cows could be considered adequate (pH > 6.0 and $N\text{-}NH_3$ > 10 mg dL^{-1}; Figure 1) for the activity of the populations of bacteria that ferment fibrous carbohydrates (Valadares & Pina, 2011), demonstrating the similarity (P>0.05) among treatments in the NDF degradability parameters of sugarcane silage (Table 3) and in the NDF digestibility of the diets (Table 2).

There was no effect (P>0.05) of including WCS on the rate of passage of fluids in the rumen, and the values obtained (Table 5) were generally lower than those reported in studies with Holstein x Gyr cows (14.4-20.4 kg day^{-1} of milk) fed tropical grasses supplemented with lipid concentrates (Ribeiro, Lopes, Gama, Rodriguez, & Morenz, 2018; Mourthé et al., 2019). According to Arieli (1998), linted WCS has been suggested to stratify in the ruminal contents and may be regurgitated with the forage and chewed during rumination. The stimulus to chewing promoted by the presence of lint in the WCS could thus alter the ruminal fill and decrease the passage rate of feed in the rumen (Bettero et al., 2013). However, there was no effect (P>0.05) of inclusion of WCS on the rates of particulate passage in the gastrointestinal tract of cows (Table 5). These results are in agreement with the absence (P>0.05) of effects of the treatments on NDF intake and digestibility (Table 2) and on the degradability parameters of DM and NDF of sugarcane silage (Table 3). In a study carried out with lactating cows that were fed with 55% corn silage-based diets, Bettero et al. (2013) also did not observe an effect of the inclusion of 10% WCS in the diet on the ruminal particulate passage rate of the corn silage fiber. The average value presented by Bettero et al. (2013) was 3.6% h^{-1}, which is close to the values

estimated mainly through the Grovum and Williams (1973) model (Table 5). Corroborating the results reported by Lopes et al. (2008) and Ribeiro et al. (2018), the ruminal and postruminal particulate passage rates obtained from the adjustment of the fecal chromium excretion data to the Grovum and Williams (1973) model were, respectively, higher and lower than those estimated from the Dhanoa et al. (1985) model.

The results of milk yield and composition from the present work were presented in a companion paper by Costa et al. (2011), in which the WCS was reported to not affect the milk yield and milk protein, fat and total solids content (P>0.05). WCS promoted generalized reduction (P<0.05) in the contents of *de novo*-synthesized FAs (Table 6). Comparing the control ration with the 15% WCS ration, the reductions (P<0.05) in the milk fat contents of lauric, myristic and palmitic acids were 49%, 39% and 14%, respectively. The reduction in the milk fat contents of *de novo*-synthesized FAs in response to WCS inclusion is also well documented (Chen et al., 2008; Dayani et al., 2011), and a hypothesis to explain it concerns the preferential incorporation of mono- and polyunsaturated FAs into the triglycerides of the milk fat (Shingfield, Bernard, Leroux, & Chilliard, 2010). This hypothesis is supported by the negative correlations (r = -0.65 to -0.93, P<0.01) between the sum of the milk fat contents of *de novo*-synthesized FAs (C4:0 to C16:0) versus those of several C18-unsaturated FAs (*e.g.*, oleic, elaidic, *trans*-10 18:1). There was also a high negative correlation (r = -0.90, P<0.0001) between the sum of the milk fat contents of *de novo*-synthesized FAs versus milk fat stearic acid content. Kadegowda, Bionaz, Piperova, Erdman and Loor (2009) observed that *trans*-10 C18:1 reduced the expression of the FA synthetase enzyme by 61%, while stearic acid promoted a reduction of 56% and 69% in the expression of acetyl-CoA carboxylase and FA synthetase enzymes, respectively, which are key enzymes for *de novo* FA synthesis in the mammary gland. According to Kadegowda et al. (2009), since there is no endogenous synthesis of stearic acid, which is primarily taken up from the plasma, the mammary gland cells reduce *de novo* FA synthesis in favor of FA esterification reactions for milk fat triglycerides. Another factor that may have contributed to the reduction in *de novo* FA synthesis in the mammary gland is lower plasma concentrations of the precursors β-hydroxybutyrate and mainly acetate (Shingfield et al., 2010). In the control treatment, the ruminal acetate content was higher (P<0.05) than that of the 15% WCS diet only at 4 and 10 hours after the provision of the morning ration (data not shown).

Despite the absence of an effect (P>0.05) on NDF *in vivo* digestibility (Table 2) and on NDF *in situ* ruminal degradability (Table 3), WCS inclusion in the diet promoted a generalized linear reduction (P<0.001) in the milk content of the major OBCFA (Table 6). OBCFAs originate from the digestion and absorption of lipids synthesized by ruminal bacteria and from the *de novo* synthesis of C13:0, C15:0 and C17:0 FAs (Kliem & Shingfield, 2016). The substitution of ground corn and cottonseed meal by the EE present in WCS already reduces the amount of fermentable substrate available for the ruminal microbiota, thereby limiting its development and reducing microbial protein synthesis (Almeida et al., 2016).

WCS inclusion in the diet promoted an intense ruminal BH. The FA composition of the milk fat confirms this possibility, as there was a generalized increase (P<0.05) in the contents of known intermediates of ruminal BH of oleic, linoleic and α-linolenic acids (Shingfield et al., 2010). For example, in relation to the control treatment, the *cis*-12 C18:1, elaidic acid, *trans*-10 C18:1, *trans*-12 C18:1 and *trans*-16 C18:1 contents in the milk fat of the cows that received 15% WCS increased by 82%, 33%, 30%, 131% and 167%, respectively (Table 7). In addition, there was a linear increase (P=0.0008) in the stearic acid content in milk fat (Table 6). Notably, stearic acid is the common end product of the main ruminal BH pathways

(Shingfield et al., 2010). Comparing the control ration with the 15% WCS ration, this increase in the stearic acid content in milk fat was 89%, which indicates that the unsaturated-C18 FAs protected inside the WCS were slowly released in the ruminal environment, not exceeding the hydrogenating capacity of the cellulolytic bacteria in the rumen. The linear increases (P<0.01) on the C18:0/*trans*-11 C18:1, C18:0/*trans*-10 C18:1 and C18:0/*trans*-9 C18:1 ratios in response to inclusion of WCS in the diet (data not shown) are also indicative of nonlimitation to complete BH of unsaturated-C18 FAs in the rumen.

WCS promoted a linear increase (P<0.0001) in the content of oleic acid in the milk fat in response to WCS (Table 7). In relation to the control treatment, the oleic acid content of milk fat of the cows that received 15% WCS increased by 55%. The increase in the milk fat content of stearic and oleic acids in response to WCS supplementation is well documented (Chen et al., 2008; Dayani et al., 2011). There was a quadratic effect (P=0.0348) of WCS on the SCD activity for the oleic acid/stearic acid pair (Table 9), indicating some degree of inhibition of this enzyme. The negative correlations between the SCD activity for the oleic acid/stearic acid pair *versus* the milk fat content of *trans*-10 C18:1 (r = -0.72; P<0.01) and *trans*-10, *cis*-12 CLA (r = -0.59, P<0.05) can be considered indicative that these FAs may have partially modulated the synthesis of oleic acid in the mammary gland. These results corroborate those presented by Kadegowda et al. (2009), who reported that *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA reduced the expression of SCD enzyme by 100% and 357%, respectively. These results indicate the importance of the contribution of oleic acid that escaped from the ruminal BH and was captured from the plasma by the mammary gland for its secretion in milk. Esterification of oleic acid at the *sn*-3 position of triacylglycerol constitutes an important mechanism for controlling the melting point and fluidity of milk fat (Shingfield et al., 2010).

There was no effect (P>0.05) of inclusion of WCS on the milk fat content of vaccenic acid (Table 7). Since vaccenic acid is the precursor for the synthesis of 70-95% of the total amount of rumenic acid secreted in bovine milk (Kliem & Shingfield, 2016), there was also no effect (P>0.05) of WCS supplementation on the SCD activity for the rumenic/vaccenic acid pair (Table 9) and on rumenic acid milk fat content (Table 8). The absence of response to WCS supplementation on the bovine milk fat contents of vaccenic and rumenic acids was also reported (Dayani et al., 2011). The negative correlations between the SCD activity for the rumenic/vaccenic acid pair *versus* the milk fat content of *trans*-10 C18:1 (r = -0.70; P<0.01) and stearic acid (r = -0.75, P=0.0012) can be considered indicative of some degree of inhibition of this enzyme. Notably, stearic acid is a substrate that competes with vaccenic acid for the action of SCD, and Kadegowda et al. (2009) reported that *trans*-10 C18:1 reduced the expression of the SCD enzyme.

According to Martins et al. (2016), the pair of FAs *cis*-9 C14:1/myristic is the one that best represents the SCD activity in the mammary gland, since myristic acid originates almost exclusively during *de novo* synthesis in the mammary gland. The SCD activity for this pair of FAs was linearly reduced (P=0.0010) in response to WCS inclusion (Table 9). Two cyclopropene FAs, malvalic and sterculic, that are found in cottonseed at approximately 607-1,278 μg g^{-1} and 517-842 μg g^{-1}, respectively (Obert, Hughes, Sorenson, McCann, & Ridley, 2007), are also inhibitors of desaturase enzymes in lactating cows (Cook, Scott, Mills, Fogerty, & Johnson, 1976). However, based on the results presented by Cook et al. (1976), Coppock et al. (1987) suggest that during the feeding of WCS, most of the cyclopropene FAs are probably saturated. Another factor that may have reduced the activity of SCD was the administration of Co-EDTA, since according to Leskinen et al. (2016), Co-EDTA partially inhibits or decreases the

desaturation rate of SCD enzyme in the mammary gland of cows. The milk fat contents of vaccenic (Table 7) and rumenic acids (Table 8) can be considered low, being smaller (1.07-1.40 g 100^{-1} g FA) or near (0.40-0.56 g 100^{-1} g FA), respectively, than those observed in the milk fat of cows fed diets based on fresh sugarcane or on sugarcane silage not supplemented with lipid concentrates (Meneses et al., 2015; Martins et al., 2016; Souza et al., 2019).

Mohammed et al. (2009) reported that ~79% of the production of rumenic acid in bovine milk was explained by α-linolenic acid intake. None of the ingredients used in the diets, including WCS (Chen et al., 2008) and sugarcane silage (Martins et al., 2016), are sources of α-linolenic acid. Thus, the two main unsaturated FAs consumed were oleic acid and, mainly, linoleic acid. The absence of an effect (P>0.05) on the inclusion of WCS in the diet on milk fat contents of linoleic and α-linolenic acids indicates that there was an extensive BH of them, which was confirmed by the linear increases (P<0.05) in milk fat contents of stearic acid (Table 6) and several other FA intermediates of the ruminal BH processes (Table 7). The linear increase (P=0.0180) in the *trans*-11 C18:1/*trans*-10 C18:1 ratio in response to WCS inclusion (data not shown) can be considered indicative of a lower ruminal production of vaccenic acid and/or that the extent of its BH was greater than that of *trans*-10 C18:1. Thus, there was less vaccenic acid available to the mammary gland to capture from the plasma for its secretion in the milk fat and for its desaturation to rumenic acid. In addition, the quadratic effect (P=0.0383) of *trans*-10, *cis*-12 CLA (Table 8) and the linear increase (P=0.0365) of *trans*-10 C18:1 contents in milk fat (Table 7) indicate that WCS diets caused some adaptation in the bacterial community of the rumen, thereby increasing the ability of BH by increasing the number of competent bacteria and/or their activity, which resulted in an increase in *trans*-10 BH when the efficiency of the *trans*-11 pathway of linoleic acid BH was reduced (Zened, Enjalbert, Nicot, & Troegeler-Meynadier, 2012).

WCS improved the nutritional quality of milk fat, since there was a linear reduction in AI and TI (P<0.0001) and an increase (P=0.0006) in the h/H FA ratio (Table 10). This effect can be attributed to the decrease in the milk fat contents of the hypercholesterolemic lauric, myristic and palmitic FAs (Table 6) and the concomitant increase in oleic acid content (Table 7), which is associated with beneficial effects on cardiovascular health (FAO, 2010), and specifically considering TI, also due to the increase in stearic acid content in milk fat (Table 6). The absence of an effect (P>0.05) of inclusion of WCS in the diet on the ω-6/ω-3 FA ratio was attributable to the similarities (P>0.05) in the contents of Σ ω-6 *cis* and Σ ω-3 *cis* FAs in milk fat (Table 8).

Conclusions

The inclusion of up to 15% whole cottonseed in a sugarcane silage-based diet did not alter the digestibility of nutrients, the rumen degradability of the fiber, or the kinetic flow of the digesta in the gastrointestinal tract of Holstein x Gyr cows. However, the nutritional quality of milk fat was improved by dietary whole cottonseed inclusion as a result of an increased content of oleic acid and a concomitant reduction in the contents of hypercholesterolemic lauric, myristic and palmitic acids.

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