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Performance of lactating dairy cows fed sunflower or corn silages and concentrate based on citrus pulp or ground corn

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ABSTRACT - This study aimed to evaluate the effect of diets containing sunflower or corn silages and concentrate based on citrus pulp or ground corn on intake, apparent digestibility, feeding behavior, microbial protein production, and production, composition, and fatty acid profile of milk from dairy cows. Eight multiparous lactating Holstein cows (586±61 kg live weight; 25.0±4.0 kg daily milk yield at 80 to 120 days in milk) were randomly assigned to a double 4 × 4 Latin square design with a 2 × 2 factorial array. The experimental diets were: sunflower silage + citrus pulp-based concentrate; sunflower silage + ground corn-based concentrate; corn silage + citrus pulp-based concentrate; and corn silage + ground corn-based concentrate. The dry matter intake was highest for diets containing sunflower silage and lowest for diets with citrus pulp. Sunflower silage provided the highest intakes of crude protein, neutral detergent fiber, and ether extract. Except for the ether extract, the type of forage and carbohydrate did not influence the apparent nutrient digestibility. The forage and carbohydrate sources did not influence the feed eating time, but animals fed sunflower silage showed decreased rumination time and chewing activity. The microbial protein production was not altered with the diets. No differences were observed for milk production or composition, except for the milk urea nitrogen and lactose concentration. The type of forage influenced the milk fatty acid profile, to which corn silage presented higher values for fatty acids up to a 17-carbon chain length. The inclusion of sunflower silage and citrus pulp, compared with corn silage and ground corn, alters dry matter intake and ingestive behavior, but maintains milk production and composition with satisfactory characteristics of the milk fatty acid profile, providing an alternative feed for dairy cows.

Key Words: digestibility, fatty acids, intake, milk composition

Introduction

The ensiling process is an efficient practice during periods of low forage production, providing good quality roughage for ruminant feeding. Corn has favorable characteristics for silage production because it offers a good yield of dry matter (DM) per hectare and high nutritional value (Viana et al., 2012). However, the technology used in corn silage demands considerable costs in technical and financial resources (Sousa et al., 2009). Sunflower silage is a potential alternative, which can replace corn in areas where weather conditions or time of year limits the growth of the corn crop (Viana et al., 2012).

The effects of using various silages on animal performance depend on the quality of the silage and nutrient availability (Mizubuti et al., 2002). The main factors responsible for the conversion of forage into animal products are intake, digestibility, and the efficiency of utilization of digestible energy (Waldo and Jorgensen, 1981).

Likewise, the non-fibrous carbohydrates (NFC) are a primary source of energy used to meet the requirements of lactating dairy cows, particularly high-producing animals. However, not all sources of NFC support similar production performance rates. Understanding how carbohydrates are arranged in the total ration system and how they differ regarding the nutrients provided to the animal may provide greater information on how to include them in the formulation of diets.

Feeding behavior and rumen function are interrelated and metabolism is highly dependent on the processes occurring in the rumen. Consequently, feeding behavior and rumen function influence both the nutrients entering the blood and those available to the udder for milk production

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(Giga-Reverdin et al., 2014). The interrelationship between the physical and chemical properties of food, amount of food consumed, microbial population of the rumen, and ruminal removal rate of the products of digestion and fermentation and indigestible residue should constantly be evaluated as a means of improving animal production (Chalupa, 1977).

The present study aimed to evaluate intake, apparent digestibility, feeding behavior, microbial protein production (MPP), and production, composition, and fatty acid profile of milk from dairy cows fed diets containing sunflower or corn silage and concentrates based on citrus pulp or ground corn.

Material and Methods

The sunflower (M734 genotype; a simple hybrid used for oil production) silage was performed when the plants reached the R9 stage, using a conventional forage harvester coupled to a tractor. Two surface silos were made, with dimensions of 16 m long × 4 m wide, which were closed on the same day. A tractor was used to compact the deposited material in the silos. The seeding rate for the sunflower crop was approximately 45,000 plants per ha, with a spacing of 0.90 m between rows and 0.25 m between plants.

Corn (AG 1051) silage was performed when the grains were in the dough stage, using the same equipment mentioned above, but the forage was stored in trench-type silos. All procedures carried out on the sunflower silage were repeated for the corn silage, except the closing of the trench-type silos, which, on average, occurred after five days (Table 1). The seeding density was approximately 55,000 plants per hectare, with a spacing of 0.90 m between rows and 0.20 m between plants. The forage was chopped at a theoretical length-of-cut of 1.9 cm.

Eight multiparous lactating cows, with an average body weight of 586±61 kg and an average daily yield of 25.0±4.0 kg of milk (80 to 120 days in milk), were used in a double 4 × 4 Latin square design with a 2 × 2 factorial array. The experiment lasted 84 days, with four 21-day periods that consisted of 14 days for adaptation and seven days for data collections. The animals remained confined separately in tie-stall facilities with free access to water.

Table 1 - Chemical composition of sunflower and corn silages

Item	Sunflower silage	Corn silage
Dry matter (g kg^{-1})	300.0	396.0
Crude protein (g kg^{-1} DM)	100.0	86.0
Neutral detergent fiber (g kg^{-1} DM)	530.0	562.0
Acid detergent fiber (g kg^{-1} DM)	430.0	312.0
Ether extract (g kg^{-1} DM)	142.0	37.0
pH	5.56	4.01
N-NH_3/TN	17.54	7.05

DM - dry matter; TN - total nitrogen.

Four diets, formulated to meet the animal requirements according to the NRC (2001), were evaluated: sunflower silage + citrus pulp-based concentrate (SSP); sunflower silage + ground corn-based concentrate (SSC); corn silage + citrus pulp-based concentrate (CSP); and corn silage + ground corn-based concentrate (CSC) (Table 2). The roughage:concentrate ratio adopted was 50:50, on a total DM basis. Diets were isoproteically balanced and, depending on the evaluated treatment, contained sunflower or corn silages as the only roughage source.

The diets were supplied twice daily (07.00 and 17.00 h), as a total mixed ration, in sufficient amounts to allow for 10% as orts. The daily voluntary intake was calculated by subtracting the amount of feed supplied in the two meals by the orts weighed in the following morning before the first meal of the day. Each day, after the supplied feed and orts had been homogenized in the trough, samples were collected, conditioned in identified plastic bags, and stored at –14 °C before analyses.

Feces were collected directly from the rectal ampulla between the 15th and 20th days of the experimental period, with two samples collected in the morning and afternoon. Sampled feces were conditioned in plastic bags, identified, and frozen at –14 °C, generating composite samples per animal in each period. The apparent digestibility coefficient of the nutrients was determined by the indigestible acid detergent fiber (iADF) as an internal indicator on the samples of supplied feed, orts, and feces at 144 h (Craig et al., 1984; Cochran et al., 1986). After incubation, the acid detergent fiber insoluble residue was determined according to Van Soest et al. (1991).

Table 2 - Chemical composition (g kg^{-1}) of experimental diets, on dry matter basis

Item	Diet			
	SSP	SSC	CSP	CSC
Dry matter	452.0	465.0	405.0	400.0
Organic matter	83.0	71.0	65.0	78.0
Crude protein	159.0	143.0	148.0	151.0
Neutral detergent fiber	447.0	457.0	478.0	461.0
Acid detergent fiber	333.0	312.0	336.0	310.0
Non-fibrous carbohydrates[1]	310.0	312.0	332.0	350.0
Ether extract	103.0	102.0	26.0	28.0
Ca	16.0	13.0	11.0	13.0
P	4.0	4.0	4.0	5.0

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate.

[1] NFC% = 100% − [CP% + (NDF% − NDFCP%) + EE% + Ash%), according to Sniffen et al. (1992), in which NFC = non-fibrous carbohydrates; CP = crude protein; NDF = neutral detergent fiber; NDFCP = neutral detergent-insoluble CP; and EE = ether extract.

At the end of the experiment, samples of supplied feed, orts, and feces were thawed at room temperature and pre-dried in a forced-air oven at 65 °C for 72 h. Afterwards, they were ground in a Thomas Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia PA, USA), with 1-mm sieves. The samples were analyzed for DM contents (AOAC 1990; method no. 930.15), ash (AOAC 1990; method no. 924.05), crude protein (CP) (AOAC 1990; method no. 984.13), and ether extract (EE) (AOAC 1990; method no. 920.39). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest et al. (1991). The difference between the DM and ash contents determined the organic matter (OM).

The percentage of NFC in the diets was calculated according to the equation proposed by Sniffen et al. (1992): NFC% = 100% – [CP% + (NDF% – NDFCP%) + EE% + Ash%], in which NDFCP = neutral detergent-insoluble CP.

Fecal production (FP) and the apparent digestibility coefficient (AD) were calculated through the following expressions (Church, 1988): FP (g DM day^{-1}) = iADF intake (g day^{-1})/concentration of iADF in feces (g gDM^{-1}); and AD (g kg^{-1}) = [nutrient intake (kg day^{-1}) – nutrient excreted in feces (kg day^{-1})]/nutrient intake (kg day^{-1}) × 100.

The feeding behavior was determined between the 20th and 21st days of each experimental period, by an intermittent (every 5 min) visual observation of the eating activity of each animal, for 24 h, consecutively. Feeding behavior was evaluated based on the records of the time spent on the activities of rumination, idleness, and intake of water and food. The chewing times were determined as the sum of the feed intake and rumination times (min day^{-1}). For the chewing time (min day^{-1}) per unit of DM or NDF consumed, the average intake values from each period were considered (Johnson and Combs, 1991). During the milking period, the chewing activity was not evaluated.

On the 18th day of each experimental period, a urine spot collection was performed by massaging the vulva to measure creatinine and purine derivatives. A 5-mL aliquot of urine was diluted in 45 mL of a solution containing sulfuric acid (0.036 N) to ensure the sample remained below pH 3, thus, preventing the bacterial destruction of purine derivatives and uric acid precipitation. The identified samples were immediately frozen at –14 °C.

Creatinine and uric acid were determined using commercial kits (Labtest®), by the end-point method, with picrate and acidifier or uricase and hydrogen peroxide, respectively. Allantoin concentrations were determined according to the technique described by Chen and Gomes (1992). The purine derivatives (mmol day^{-1}) were calculated by the sum of allantoin and uric acid in the urine. The estimative of microbial protein production was obtained by multiplying the synthesis of microbial nitrogen by 6.25.

Animals were milked twice daily, at 06.00 and 16.00 h. Milk yields were obtained by consecutive milking sessions performed on the 19th, 20th, and 21st days of each experimental period. Four samples of milk were collected in successive milking sessions to determine the fat, protein, lactose, urea nitrogen, total solids, and solids-not-fat contents. To preserve the 50-mL samples, 10 mg of bronopol (2-brono 2-nitropropane 1,3-diol) was added to each container that was then refrigerated at 4 °C.

The milk fat, protein, lactose, total solids, and solids-not-fat contents of the samples were determined by near-infrared, using a Bentley 2000 machine (Bentley Instruments, Chaska, USA). Milk was corrected for 4% fat, according to the NRC (1989), as follows: 4% CMY = 0.4 × milk yield + 15 × (%fat 100^{-1}) × milk yield, in which CMY = corrected milk yield.

The urea nitrogen concentration of the samples was analyzed by the enzymatic colorimetric method, using a ChemSpec 150 device (Bentley Instruments, Chaska, USA).

Individual, homogenized samples of the milk collected in the four above-mentioned milking sessions were used to determine the fatty acid profile by liquid gas chromatography according to the extraction and methylation technique described by Chouinard et al. (1999).

The variables were tested for normality and homoscedasticity using Lilliefors (1967) and Cochran (1952) tests. The PROC MIXED procedure of SAS (Statistical Analysis System, version 6.12) was used for the statistical analyses.

The effects of the fed diets containing sunflower or corn silage and concentrates based on citrus pulp or ground corn on intake, apparent digestibility, feeding behavior, purine derivative concentration, microbial protein production, and production, composition, and fatty acid profile of milk were evaluated according to the following statistical model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_k + s_l + c_m + (sc)_{lm} + a(\alpha)_{ij} + e_{ijklm},$$

in which Y_{ijklm} = response of j animal in the k period within i square under diet lm; μ = overall mean; fixed effects: α_i = square effect (i = 1, 2); β_k = effect of the k period (k = 1 to 4); s_l = silage effect (l = sunflower or corn silage); c_m = carbohydrate effect (m = concentrates based on citrus pulp or ground corn); $(sc)_{lm}$ = interaction between silage and carbohydrate; random effect: $a(\alpha)_{ij}$ = cow effect into the square, j = 1 to 8; and e_{ijklm} = random error.

Results

No significant interaction between the various sources of forage and carbohydrate was observed for daily nutrient intake. However, the dry matter (DMI) and organic matter intake, in kg, were influenced by the type of roughage and carbohydrate, when considered separately (P<0.05) (Table 3). The observed mean values of DMI were higher for the treatments containing sunflower silage (17.40 kg for sunflower silage and 15.15 kg for corn silage) and lower for the diets with citrus pulp (15.55 kg) relative to the diets containing ground corn (16.9 kg). For NDF intake (kg), the sunflower silage provided higher values than the corn silage.

The EE intake was higher for animals fed diets containing sunflower silage than corn silage. This result was possibly due to the higher concentration of this nutrient in the referred roughage (Table 2), but without negatively influencing DMI or the intake of the fibrous fractions.

The apparent nutrient digestibility was not influenced by the experimental diets, except for EE digestibility that presented the highest values when the animals were fed sunflower silage (P>0.05) (Table 4).

There were no interactions between forage and carbohydrate sources for ingestive behavior (Table 5). The eating time was not influenced by the diets. On average, the cows spent 220 min of the day consuming feed and approximately twice as much time ruminating (465 min). Animals that consumed corn silage presented higher rumination time compared with those fed sunflower silage, which resulted in more time spent chewing (P<0.01). Also, the animals fed citrus pulp spent more time chewing than those fed ground corn (717.03 vs. 659.28 min day^{-1}, respectively). The results observed in time spent chewing were reflected in the idle time.

Except for ingestion time per kg of NDF (P = 0.07), there were differences among the diets for ingestion, rumination, and chewing activity time per kg of DM and NDF consumed (P<0.05). Cows fed citrus pulp spent more time ingesting, ruminating, and chewing (total time) per kg of DM and NDF than did cows fed ground corn.

No interaction was observed between the different types of forage and carbohydrates for the concentrations of allantoin, uric acid and creatinine, the allantoin:creatinine ratio, or the microbial protein production (Table 6). The allantoin:creatinine ratio varied from 2.47 (sunflower silage + ground corn) to 3.69 (corn silage + ground corn), with

Table 3 - Average daily intake of diet constituents of lactating cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Nutrient	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
DM (kg)	16.40	18.40	14.70	15.60	0.64	<0.01	0.03	0.41
DM (g kg^{-1} LW)	28.50	31.70	25.00	26.70	0.12	<0.01	0.05	0.54
OM (kg)	15.10	7.00	14.00	15.00	0.59	0.01	0.03	0.47
CP (kg)	2.70	2.60	2.30	2.50	0.11	0.03	0.57	0.19
NDF (kg)	7.10	8.50	6.70	7.00	0.38	0.04	0.08	0.10
NDF (g kg^{-1} LW)	12.30	14.60	11.90	12.10	0.07	0.06	0.08	0.15
NFC (kg)	6.40	7.01	5.75	6.01	0.55	0.15	0.44	0.76
EE (kg)	1.74	1.96	0.37	0.39	0.09	<0.01	0.17	0.30

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; DM - dry matter; LW - live weight; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; NFC - non-fibrous carbohydrates; EE - ether extract; SEM - standard error of the mean.

[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

Table 4 - Apparent nutrient digestibility (g kg^{-1}) obtained for the experimental diets of lactating cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Item	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
Dry matter	589.5	637.4	572.9	615.2	4.01	0.92	0.36	0.47
Organic matter	598.3	640.7	587.4	619.7	3.26	0.63	0.27	0.89
Crude protein	490.7	463.7	470.8	461.0	2.48	0.81	0.70	0.86
Neutral detergent fiber	425.8	496.5	393.7	416.2	4.27	0.20	0.29	0.58
Acid detergent fiber	380.0	354.1	351.7	362.1	3.62	0.78	0.83	0.62
Ether extract	754.4	728.6	573.4	525.3	3.52	<0.01	0.30	0.75

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; SEM - standard error of the mean.

[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

the lowest values observed in the animals receiving diets with sunflower silage (P<0.01). Thus, the relatively higher allantoin:creatinine in the diets with corn silage compared with sunflower silage reflects the excretion of allantoin, which suggests adequate microbial growth. The microbial protein production did not present differences among the evaluated diets (P>0.05).

No differences were observed for the milk production and composition parameters between the forages and types of carbohydrates or the interaction between them (Table 7),

Table 5 - Ingestive behavior of lactating cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Item	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
Eating (min day^{-1})	228.85	209.69	237.04	215.50	12.03	0.57	0.11	0.92
Idle (min day^{-1})	729.71	782.86	639.06	705.68	22.54	<0.01	0.02	0.77
Rumination (min day^{-1})	446.35	410.10	521.81	483.26	18.83	<0.01	0.06	0.95
Water intake (min day^{-1})	35.09	37.35	42.10	35.56	5.69	0.65	0.71	0.45
Chewing (min day^{-1})	675.21	619.79	758.85	698.76	23.1	<0.01	0.02	0.92
Intake								
Min kg^{-1} DMI	14.21	11.63	16.84	13.89	0.94	0.02	<0.01	0.85
Min kg^{-1} NDF intake	32.85	25.37	36.98	30.77	2.49	0.07	0.01	0.80
Rumination								
Min kg^{-1} DMI	27.82	23.01	36.60	31.31	1.94	<0.01	0.02	0.90
Min kg^{-1} NDF intake	64.54	50.07	79.50	69.14	5.00	<0.01	0.02	0.68
Chewing								
Min kg^{-1} DMI	42.03	34.63	53.45	45.20	2.60	<0.01	<0.01	0.87
Min kg^{-1} NDF intake	97.39	75.44	116.47	99.91	6.92	<0.01	0.01	0.70

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; DMI - dry matter intake; NDF - neutral detergent fiber; SEM - standard error of the mean.
[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

Table 6 - Concentrations of allantoin, uric acid and creatinine, allantoin:creatinine ratio, and microbial protein production of lactating cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Item	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
Allantoin (g L^{-1})	12.36	14.69	29.56	33.27	1.52	<0.01	0.06	0.65
Uric acid (g L^{-1})	0.38	0.49	0.44	0.38	0.04	0.57	0.61	0.08
Creatinine (g L^{-1})	4.11	7.40	8.40	9.00	1.13	0.02	0.10	0.25
Allantoin:creatinine	3.00	2.47	3.54	3.69	0.19	<0.01	0.32	0.09
MPP (g d^{-1})	1389.64	1414.95	1372.23	1356.61	149.7	0.83	0.98	0.91

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; MPP - microbial protein production; SEM - standard error of the mean.
[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

Table 7 - Milk yield and composition of lactating cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Item	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
Milk (kg day^{-1})	25.10	24.10	26.60	23.70	1.34	0.67	0.16	0.47
4% CMY (kg day^{-1})	25.40	22.30	23.20	22.50	1.32	0.94	0.10	0.54
Milk kg^{-1} DMI (kg)	1.60	1.65	1.58	1.45	0.11	0.32	0.74	0.44
Protein (g kg^{-1})	29.60	28.40	27.80	29.60	0.08	0.29	0.29	0.29
Protein (kg)	0.74	0.71	0.74	0.70	0.04	0.86	0.41	0.81
Fat (g kg^{-1})	35.50	34.10	34.60	32.70	0.15	0.48	0.31	0.87
Fat (kg)	0.89	0.82	0.91	0.79	0.06	0.88	0.11	0.63
MUN (mg dL^{-1})	15.90	13.70	15.70	13.80	0.84	0.96	0.03	0.83
Lactose (g kg^{-1})	46.10	47.30	45.70	47.50	0.04	0.77	<0.01	0.56
Total solids (g kg^{-1})	122.00	123.00	121.00	123.00	0.22	0.79	0.42	0.70

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; CMY - corrected milk yield; DMI - dry matter intake; MUN - milk urea nitrogen; SEM - standard error of the mean.
[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

except for the type of carbohydrate on the concentration of milk urea nitrogen and lactose. The addition of ground corn to the diet decreased milk urea nitrogen and enhanced the content of lactose in the milk.

In the present study, the interaction between forage and carbohydrate sources only influenced the concentration of $C_{6:0}$. The forage type changed the fatty acid profile of the produced milk, except for $C_{20:0}$ (Table 8). The sunflower silage, regardless of the type of carbohydrate used, was responsible for the decrease in the short-chain fatty acid contents, providing an increase in final production of stearic acid ($C_{18:0}$). Both sunflower silage and citrus pulp enhanced the content of rumenic acid ($C_{18:1}$ *cis*-9, *trans*-11) in the milk.

Discussion

The rumen-fill effect, the feed fermentability, the type and amount of lipids in the diet, and the nutrients that limit the maximum milk production (carbohydrates, proteins, and their constant availability) are notable dietary factors that can interfere with feed intake.

The NFC of the diets were higher for the treatments containing corn silage than sunflower silage (Table 2). Thus, the availability of the more fermentable nutrients might have influenced the intake of the corn silage-based diets. The NDF content of this silage (Table 2) remained above the minimum recommended for lactating cow diets, having a high nutritional density and containing starch-rich energy concentrates. Therefore, it is reasonable to state that the fibrous fraction of lower degradation rate and extent, associated with the type of carbohydrate and particle size of the silages, influenced the nutrient intake and, consequently, feeding behavior. Leite et al. (2006), studying the use of sunflower and corn silages for dairy cows, found that the lower apparent digestibility of NDF (30.7%) and ADF (28.4%), coupled with the EE concentration of sunflower silage, may compromise the intake when compared with corn silage.

According to Forbes (1995), the limiting factor to forage intake would be the ruminal fill provided by the fiber. The volume of the plant cell has been linked to the filling effect. Hence, this effect attributed to the lower intake of treatments containing corn silage (i.e. corn silage + citrus pulp and corn silage + ground corn) compared with sunflower silage + ground corn and may be ascribed to the interaction among filling, the rumen-distension capacity, and the energy density because no differences

Table 8 - Fatty acid profile of the milk fat from dairy cows fed sunflower or corn silage and citrus pulp or ground corn-based concentrate

Fatty acid (mg g⁻¹ of milk fat)	Diet				SEM	Significance[1]		
	SSP	SSC	CSP	CSC		Forage	CHO	Forage × CHO
$C_{4:0}$	3.17	3.60	4.28	4.30	0.16	<0.01	0.90	0.05
$C_{6:0}$	1.18	1.49	2.33	2.38	0.07	<0.01	0.13	0.04
$C_{8:0}$	0.52	0.70	1.27	1.35	0.05	<0.01	0.03	0.27
$C_{10:0}$	0.98	1.36	2.68	2.96	0.14	<0.01	0.03	0.87
$C_{11:0}$	0.07	0.11	0.28	0.29	0.01	<0.01	0.06	0.50
$C_{12:0}$	1.19	1.57	3.06	3.41	0.16	<0.01	0.03	0.79
$C_{13:0}$	0.03	0.04	0.09	0.10	<0.01	<0.01	0.12	0.83
$C_{12:1}$	0.03	0.04	0.09	0.11	<0.01	<0.01	0.07	0.67
$C_{14:0}$	5.20	6.57	10.91	11.11	0.40	<0.01	0.06	0.23
$C_{14:1}$ *cis*-9	0.37	0.48	0.94	0.97	0.05	<0.01	0.29	0.20
$C_{15:0}$	0.56	0.59	0.92	0.94	0.03	<0.01	0.73	0.77
$C_{16:0}$	16.37	18.15	33.80	33.18	0.88	<0.01	0.80	0.08
$C_{16:1}$ *cis*-9	0.87	0.82	1.60	1.54	0.07	<0.01	0.26	0.74
$C_{17:0}$	0.24	0.28	0.53	0.44	0.03	<0.01	0.58	0.09
$C_{17:1}$	0.17	0.17	0.28	0.22	0.02	<0.01	0.05	0.08
$C_{18:0}$	15.03	14.37	9.40	10.81	0.97	<0.01	0.63	0.22
$C_{18:1}$ *cis*-9	26.19	25.27	18.64	17.94	0.61	<0.01	0.57	0.31
$C_{18:1}$ *trans*-11	12.15	10.19	1.51	1.13	0.65	<0.01	0.09	0.45
$C_{18:2}$ *cis*-9 *trans*-11	1.09	0.87	0.14	0.10	0.05	<0.01	0.02	0.17
$C_{18:2}$ *cis*-9 *cis*-12	5.22	4.58	2.29	2.25	0.20	<0.01	0.16	0.20
$C_{20:0}$	0.10	0.97	0.94	0.94	<0.01	0.20	0.94	0.55

SSP - sunflower silage and citrus pulp-based concentrate; SSC - sunflower silage and ground corn-based concentrate; CSP - corn silage and citrus pulp-based concentrate; CSC - corn silage and ground corn-based concentrate; SEM - standard error of the mean.

[1] Significance (P-value, SNK test at the 5% level of probability): source of forage (Forage), source of carbohydrate (CHO), and interaction between forage and carbohydrate (Forage × CHO).

were found between the treatments regarding the time used for feeding.

The inclusion of sunflower silage increased the digestibility of EE, which can be explained by the high content of EE in this ingredient and consequently, greater intestinal uptake of fatty acids in cows fed the sunflower silage. Despite the increase in lipid content in the diet (EE average of 102.5 g kg^{-1} in the diets with sunflower silage), the digestibility of the fibrous fraction was not influenced. Pimentel et al. (2012) did not observe a decrease in DMI or digestibility of the fibrous fraction of the diet of dairy cows fed up to 73.1 g kg^{-1} of EE.

Similar results to that reported in the current study were found by Costa et al. (2011), who evaluated the supplementation of grazing cattle using corn and citrus pulp, and observed no effect of the carbohydrate sources on the intake of CP, NDF, NFC, and EE. These authors also found a similar behavior in the digestibility of nutrients to that observed in the present study.

The evaluation of purine derivatives (allantoin, uric acid, hypoxanthine, and xanthine) confirms that the nucleic acids in the duodenum are mostly of microbial origin. After intestinal digestion and absorption, these derivatives are proportionally recovered in the urine, so that their measurements constitute a reliable microbial marker (Orellana-Boero et al., 2001).

Burke et al. (2007) did not find significant differences in the urinary concentrations of allantoin or creatinine, or in the allantoin nitrogen:creatinine nitrogen ratio between grass silage, fermented whole-crop wheat, urea-treated processed whole-crop wheat, and corn silage in the diets of early lactating dairy cows. San Emeterio et al. (2000) reported an increase from 2.34 to 3.10 in this ratio, when high-moisture ensiled shelled corn was supplied to Holstein cows fed once daily, indicating an increment in rumen microbial growth. In the present study, although the allantoin:creatinine ratio was higher for diets containing corn silage than sunflower silage, the increment was not sufficient to enhance the microbial protein production. The amount of rapidly available energy in the rumen and the presence of nutrients that affect the population of bacteria and protozoa in the rumen also interfere with the conversion of dietary nitrogen to microbial protein.

Nutrition can be used as a tool to alter the milk composition, but the correlation between food constituents and milk composition is complex (Arnould et al., 2013). Despite expectations in higher milk fat content, when pectin and fiber of corn silage resulted in higher rumination per kg DM and NDF, there was no increase in this variable by type of forage or concentrate. The fermentation profile of pectin, with a higher proportion of acetic acid relative to the fermentation of starch, besides possibly maintaining a higher pH in the rumen (Bampidis and Robinson, 2006), supports the expectation of increased production of milk fat in diets containing citrus pulp. However, the change in the association between acetate and propionate appears to be an insufficient explanation for the observed fat content in the milk, in response to the variation in the dietary carbohydrate profile.

Neither forage nor carbohydrate source influenced the percentage of milk protein, showing only a low yield regarding the genetic merit of the animals and the CP intake, which were based on the recommendation of the NRC (2001). Therefore, the inclusion of sunflower or corn silage and also the presence of pectin or starch in the diet, promoted the decrease in milk protein content.

Silva et al. (2004) observed a decrease in milk and protein yields but did not find any influence of feeding sunflower versus corn silage in the diets of dairy cows, regarding the protein and fat contents of milk or milk yield (corrected for 4% fat).

In the present study, the significant increase in the dietary lipid level, with the addition of sunflower silage, probably provided an increase in the concentration of *trans*-11 and *cis*-9 *trans*-11 (rumenic acid) in the milk fat (93 and 92%, respectively), compared with the diet containing corn silage. Although the literature demonstrates that dairy cows have the ability to endogenously synthesize *cis*-9 *trans*-11 from *trans*-11 (Griinari et al., 2000), the presence of sunflower silage as a roughage source, irrespective of the type of carbohydrate, increased the $C_{18:1}$ *trans*-11 level. Hence, it can be inferred that the type of carbohydrate provided an increase in the rumenic acid levels in diets and that there was an increase in the level of this fatty acid, influenced by both sunflower silage and the citrus pulp.

The sunflower silage used in the sunflower silage + citrus pulp and sunflower silage + ground corn treatments, which differed from the carbohydrate type supplied in the concentrate, possibly increased the concentrations of $C_{18:1}$ *trans*-11 and $C_{18:2}$ *cis*-9 *trans*-11, which is explained by the higher dietary concentration of $C_{18:2}$ *cis*-9 *cis*-12, the main unsaturated fatty acid precursor of *trans*-11 and *cis*-9 *trans*-11. Considering that the sunflower silage is a source of $C_{18:2}$ *cis*-9 *cis*-12 in the diet and is associated with the effect of the high presence of lipids in the rumen, many of these might have passed from the rumen into the intestine. These lipids were then absorbed and incorporated directly into the milk fat, without interfering with the *de novo* synthesis in the mammary gland.

Conclusions

The inclusion of sunflower silage and citrus pulp as forage and carbohydrate sources, respectively, compared with corn silage and ground corn, alters dry matter intake and ingestive behavior, but maintains milk production and composition, with satisfactory characteristics of the milk fatty acid profile. Thus, diets formulated with sunflower silage and citrus pulp provide an alternative feed for dairy cows.

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