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Feeding F1 Holstein x Zebu cows with different roughages and pseudostem hay of banana trees does not influence milk yield and chemical composition of milk and cheese

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ABSTRACT
The aim of the study was to determine the effect of different roughage associated with pseudostem hay of banana trees on nutrients intake, chemical composition of milk and cheese and the fatty acid profile of F1 Holstein x Zebu cows. Eight cows F1 Holstein x Zebu were used in a replicated 4 × 4 Latin square design, each composed of four animals, four treatments and four experimental periods. Four experimental diets were used: diet 1 – sorghum silage as exclusive dietary roughage; diet 2 – sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis; SS); diet 3 – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis; EGPS); and diet 4 – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis; SCPS). The roughage: concentrate ratio in the four experimental diets was 75:25 in dry matter basis. For the chemical composition of milk and cheese, there was no difference between diets except for milk urea nitrogen which was higher with the EGPS diet. However, in the lipid fraction of milk, the EGPS diet favoured the increase of total monounsaturated fatty acids, besides increasing the concentration of conjugated linoleic acid, contributing to the improvement of some nutritional evaluation indexes of the milk lipid fraction. The pseudostem hay of banana trees associated with different roughages did not influence the chemical composition of milk and cheese of F1 Holstein x Zebu cows.

HIGHLIGHTS

- The inclusion of pseudostem hay of banana trees in the diet of F1 Holstein x Zebu cows does not change the milk yield corrected for 3.5% fat.
- Pseudostem hay of banana trees diet of lactating cows increased the concentration of omega 6 in milk.
- Pseudostem hay in the diet of lactating crossbred cows did not change the yield, flavour and appearance of the cheese.

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Introduction

In Brazil, an increase in the milk consumption and milk products, as well as the export opportunities of dairy products has required improvement of current production systems. Currently, almost 800 billion litres of milk are consumed annually in the world and Brazil produces 4.38% of this volume, being the fifth largest producer in the world (Leite 2018). However, average milk yield per cow is below the world average of 3.5 thousand litres per lactation. This is due to the variation in the nutritional value of tropical grasses throughout the year modifying the productivity of animals. The feedlot of dairy cows is a strategy to improve the productive potential of the animals due to the quality of the diet provided (Monção et al. 2020). In the semiarid region of Brazil, sorghum silage

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(*Sorghum bicolour* (L.) Moench) It is traditionally used as a roughage (fibre source) for feedlot dairy cows due to tolerance to water restriction, mechanisable and high productivity per unit area and good nutritional value (Borges et al. 2019). However, the feed cost under these conditions is high (Borges et al. 2019; Santana et al. 2019, 2020). The use of traditional ingredients or byproducts and/or agribusiness wastes available in each region in the formulation of diets is a strategy to reduce feed costs and improve system efficiency.

The banana crop (*Musa* spp.) is an activity practiced worldwide, generating large amounts of residues (waste) after harvest. Souza et al. (2010) verified that for each ton of banana processed, three tons of pseudostem with 10% of dry matter are generated. The Food and Agriculture Organisation (FAO) estimates that in 2017, 114 million tons of fruit were produced worldwide, generating large amounts of pseudostem. According to Carmo et al. (2018), the use of pseudostem hay of banana trees is an alternative for animal feed because it does not alter the dry matter intake in small ruminants nor the production of rumen short chain fatty acids and improves dry matter digestibility. However, there are no studies in the literature using pseudostem hay of banana trees in the F1 Holstein x Zebu cow diet, which account for 80% of the volume of milk produced in Brazil. For Nudda et al. (2014), feeding the cow is the main strategy to change and improve the quality of milk and dairy products. However, according to Liu et al. (2016), the milk fat composition, conjugated linoleic acid (CLA) content and its derivatives (i.e. cheese, yoghurt) can be modified with the inclusion of different fibre sources in the diet as the pseudostem hay. Several studies have already been performed evaluating the replacement of traditional roughages (i.e. sorghum silage, sugar cane, elephant grass) by agro-industry byproducts on animal performance, milk composition and quality of dairy cows showing good results (Pimentel et al. 2017; Antunes et al. 2018; Melo et al. 2018; Santiago et al. 2019). Thus, in F1 Holstein x Zebu lactating cows, it is necessary to evaluate the nutrient intake and milk and cheese composition after the inclusion of pseudostem hay in the diet associated with different roughages.

Based on the above, the objective of this study was to evaluate the effect of different roughages associated with pseudostem hay of banana trees on nutrient intake, chemical composition of milk and cheese and fatty acid profile of F1 Holstein x Zebu cows.

Materials and methods

Local, period, facilities, design and animals

The experiment was conducted at the State University of Montes Claros (Unimontes), Janaúba, Minas Gerais, Brazil. The experiment lasted 72 days, which were divided into four periods of 18 days, 14 days for the adaptation of the animals to the diets and management, and 4 for data collection and samples. The experimental design was simultaneous in two 4×4 Latin squares, being four diets (treatment), four experimental periods and four animals. Animals were kept in individual pens (3×2 m) surrounded by a smooth wire with fibre-cement tile floors. The study included eight F1 Holstein x Zebu cows, with 582 ± 21 kg of initial body weight, 80 ± 10 days of lactation at the beginning of the experiment and a mean age of 96 mo.

Experimental diets and management

Four experimental diets were used: diet 1 – sorghum silage as exclusive dietary roughage; diet 2 – sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); diet 3 – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis); and diet 4 – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis). The roughage:concentrate ratio in the four experimental diets was 75:25 in dry matter basis. The pseudostems of banana trees were collected at the Unimontes experimental farm, processed in a forage harvester, model JF-90 (JF Agricultural machinery, Itapira São Paulo Brazil), and placed in 10 cm layers for pre-drying with manual stirring once a day. After drying, they were stored in sacks and stored in a covered shed. Prior to delivery to the animals, the pseudostem hay was subjected to further processing in a stationary chopper adjusted to 2 cm to decrease the particle size. The sorghum used for silage production was *Sorghum bicolour* (L.) Moench cv. Volumax. Elephant grass, *Pennisetum purpureum* Schum cv. Roxo de Botucatu, and sugar cane, variety IAC 86-2480, were obtained at the Experimental Farm of Unimontes, managed with irrigation and harvested at two and three metres in height, respectively. Elephant grass and sugar cane were collected daily and were processed in a stationary chopper, set at 2 cm, just before the diets were supplied. Urea was used to correct the crude protein (CP) contents of the diets, and a single concentrate was used in the four experimental diets. To ensure

Table 1. Proportion of ingredients and chemical composition of experimental diets.

	Experimental diets[a] (g/kg of dry matter)			
Item	SS	SSPS	EGPS	SCPS
Pseudostem hay	0.00	223.29	223.71	220.92
Sorghum silage	747.60	521.01	0.00	0.00
Elephant grass	0.00	0.00	521.99	0.00
Sugar cane	0.00	0.00	0.00	515.48
Ground corn	156.08	156.08	156.08	156.08
Soybean meal	84.27	84.27	84.27	84.27
Urea/ammonium sulphate (9:1)	2.40	5.70	4.30	13.60
Mineral mix[b]	9.65	9.65	9.65	9.65
	Chemical composition			
Dry matter	464.50	593.00	565.90	578.50
Ashes	64.30	88.80	95.90	77.60
Crude protein	122.70	124.90	120.10	120.80
Ether extract	21.80	19.43	15.23	16.67
Total carbohydrates	791.20	766.87	768.77	784.93
Nonfibrous carbohydrates	310.84	293.82	314.65	424.09
Neutral detergent fibre	530.30	515.50	503.60	409.80
NDFap[c]	489.00	475.10	469.60	392.50
iNDF[d]	161.10	145.40	227.40	159.90
Acid detergent fibre	324.20	334.50	352.30	267.10
Lignin	120.20	122.30	135.50	99.30

[a]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis).
[b]Mineral mix, content per kg of product: calcium (128 g min), phosphorus (100 g min), sodium (120 g min), magnesium (15 g), sulphur (33 g), cobalt (135 mg), iron 918 mg), iodine (160 mg), manganese (1800 mg), selenium (34 mg), zinc (5760 mg), fluorine (1000 mg).
[c]NDFap – Neutral detergent fibre corrected for ash and protein.
[d]iNDF – Indigestible neutral detergent fibre.

the maintenance of the roughage ratio: concentrated in the total dry matter of the diets and that they were maintained isoproteic, the dry matter and CP contents of the roughages were analysed weekly. The diets were formulated according to the NRC (2001) for cows with an average of 550 kg body weight and average milk production corrected to 3.5% fat of 15 kg/day, which was given to cows twice a day at 7:00 a.m. and 2:00 p.m., in a complete diet system (total ratio mixed-TRM), which was homogenised in the trough. The feeds were weighed individually, twice daily, and the amount provided was calculated based on the leftovers, which represented 5% of the total dry matter supplied. The leftovers (refusals) were weighed daily. The cows were milked twice a day at 8:00 a.m. and at 3:00 p.m., and the presence of the calf was used to stimulate the descent of the milk.

Proportion of ingredients and the chemical composition of diets

At the end of each experimental period, a sample composed of animal (two animals by treatment) was made, being pre-dried in a forced ventilation oven at 55 °C for 72 h. Samples were milled with sieve knives with 1 mm diameter sieves for chemical–bromatological analysis. Samples of the supplied ingredients, leftovers and faeces were analysed for dry matter (method INCT-CA G-001/1 and G-003/1), crude ash and organic matter (method INCT-CA M-001/1, CP $N \times 6.25$; method INCT-CA N-001/1), and ether extract (EE; method INCT-CA G-005/1); the neutral detergent fibre was corrected for ash and protein (using heat-stable alpha-amylase without sodium sulphite) (NDFap; method INCT-CA F-002/1); and the acid detergent fibre (ADF; method INCT-CA F-003/1) was determined as described by Detmann et al. (2012). Non-fibrous carbohydrates (NFC) were estimated according to Detmann et al. (2012). The total digestible nutrients (TDN) were estimated using the formula proposed by the NRC (2001). The proportion of the ingredients and the chemical composition of the experimental diets can be seen in Tables 1 and 2.

Nutrients intake, milk yield and milk composition

The dry matter intake was determined by the difference between the amounts of offered food and the leftovers. During the last four days of each experimental period, the milk yields (MY) per cow were recorded. The 3.5% fat corrected milk yield was calculated using the equation proposed by Sklan et al. (1992); $MY_{3.5\%} = MY \times (0.432 + 0.163 \times \text{fat content})$.

Additionally, during the last four days of each period, milk samples were taken from each animal, and a pool of morning and afternoon milking samples was made in proportion to the amount produced. Milk samples (50 mL) were added to flasks containing the Bronopol preservative and homogenised for further analysis. The total dry extract (TDE), dry extract (DE), milk urea nitrogen (MUN), casein, and casein as a percentage of total protein were determined via infrared analysis, and somatic cell counts (SCC) were determined via flow cytometry.

To determine the milk fatty acid profile, transmethylated samples (trans-methylation procedure) were analysed by gas chromatography (Focus GC-Finnigan) with a flame ionisation detector and a capillary CP-Sil 88 column (Varian) that was 100 mm in length and had a 0.25 μm internal diameter and 0.20 μm film thickness. Fatty acids were identified by comparing the retention times of the methyl esters of the samples with those of butter fatty acid standards. The fatty acids were quantified by normalising the peak areas of the methyl esters. The results of the fatty acids were expressed in mg/g fat. The nutritional

Table 2. Chemical composition of the ingredients (g/kg of dry matter) used in the formulation of the experimental diets.

Item[a]	Pseudostem hay	Sorghum silage	Elephant grass	Sugar cane	Ground corn	Soybean meal
	g kg^{-1} of dry matter					
Dry matter	888.30	317.10	265.30	289.40	897.20	914.00
Ashes	170.60	61.50	74.90	40.10	15.40	72.30
Organic matter	829.40	938.50	925.10	959.90	984.60	927.70
Crude protein	47.00	76.50	74.30	29.90	104.20	512.60
Ether extract	9.60	19.90	11.80	14.80	35.00	17.30
Total carbohydrates	772.80	842.00	839.10	915.30	845.30	397.90
Non-fibrous carbohydrates	232.50	240.00	247.70	470.80	678.40	261.70
Neutral detergent fibre	600.00	665.80	643.10	464.30	185.80	236.80
NDFap	540.30	602.00	591.40	444.50	166.90	136.20
iNDF	131.20	201.10	357.30	228.90	55.40	19.10
Acid detergent fibre	461.60	416.10	450.00	287.70	31.40	86.30
Lignin	164.20	154.90	180.00	111.10	15.40	19.40

[a]NDFap, Neutral detergent fibre corrected for ash and protein; iNDF, Indigestible neutral detergent fibre.

quality of the milk lipid fraction was evaluated by the fatty acid composition data, using the following calculations: Atherogenic index (AI) = {(C12:0 + (4 × C14:0) + C16:0)}/(Σ monounsaturated fatty acids (MUFA) + Σω6 + Σω3) and thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/{(0.5 × Σ MUFA) + (0.5 × Σω6 + (3 × Σω3) + (Σω3/Σω6)}, according to Ulbrich and Southgate (1991); the ratio of hypocholesterolemic fatty acids to hypercholesterolemic fatty acids = (C14:0 + C16:0)/(monounsaturated + polyunsaturated) and desirable fatty acids (DFA) = (unsaturated + C18:0), according to Costa et al. (2008); and the ratio of polyunsaturated to saturated fatty acids and the ratio of ω6 to ω3 (Costa et al. 2008).

On the last day of each experimental period (four periods), Minas Frescal cheese was made from milk for each treatment (four treatments), totalling 16 cheese samples at the end of the experiment. For the manufacture of cheese 3 L were used according to the following production: The milk from each experimental diet, separately, was weighed, filtered and subjected to pasteurisation (65 °C for 30 min); the milk was cooled to 39 °C, at which time calcium chloride (40 mL/100 L) and rennet (30 mL/100 L) were added, diluted equally with filtered water; after 40 to 60 min, milk coagulation occurred; the dough was cut with a 1.5–2 cm diced stainless steel knife, mixing the stirring and the rest to promote desorption; the dough was put into plastic forms and salted (700 g/100 L refined white salt); the cheeses were cooled to a temperature of 4 °C for approximately 12 h; The cheeses were removed from the moulds, packaged, weighed on a digital scale for yield determination and reserved for further analysis of chemical and sensory composition.

The yield for cheese moisture content was calculated considering a value of 57% as a reference for Minas Frescal cheese moisture, according to the equation: Cheese yield (kg/kg) = milk volume (L) × (100 − % target moisture)/(kg cheese × solids content (%) (Furtado 2005).

To determine the chemical characteristics of the cheese, the following analyses were performed: pH, using the Tecnopon digital peameter; fat percentage by the Gerber method; crude protein by the kjeldahl method; mineral residue fixed by the disposal of organic matter at a temperature of 550 °C; total dry extract by evaporation of sample water using the greenhouse at 105 °C and humidity was determined by subtraction of total solids; and water activity (Aw) using an AquaLab® Aw metre.

Sensory analysis of Minas Frescal cheese was performed according to the acceptance method proposed by Meilgaard et al. (1999), with untrained tasters for cheese evaluation. The analysis took place in the four experimental periods, with 25 tasters in each period. The cheese samples were cut into cubes, weighing around 25 g and identified with numerical codes, in disposable cups. The test took place simultaneously with all samples served with their respective codes and were classified by tasters who 1 to sample less accepted and 9 for the most accepted for the acceptance of evaluation in terms of appearance, consistency, flavour and overall impression of cheeses. The preference ranking methodology described followed by Meilgaard et al. (1999). Sum of the orders obtained from the testers to each of the samples.

Statistical analysis

Data were evaluated by analysis of variance using the MIXED procedure of SAS, version 9.0 (SAS Institute 2008, Inc., Cary, NC, USA). Data normality (Shapiro–Wilk test at 5% probability) was verified by the UNIVARIATE procedure in SAS. The statistical model used for analyses was $Y_{k(ij)} = \mu + T_k + P_i + A_j + \text{IBW} + e_{k(ij)}$, where $Y_{k(ij)}$ is the observation concerning the treatment "*k*" within period "*i*" and with animal "*j*";

Table 3. Intake and milk yield of F1 Holstein/Zebu cows fed diets with different roughages associated with pseudostem hay of banana trees.

Item[a]	Diets[b] SS	SSPS	EGPS	SCPS	SEM[c]	p Value[d]
Intake, % BW						
Dry matter	2.87 a	2.86 a	2.55 b	2.51 b	0.08	<.01
Crude protein	0.30 b	0.34 a	0.26 b	0.28 b	0.01	<.01
Nonfibrous carbohydrates	0.86 b	0.89 b	0.81 b	1.03 a	0.03	<.01
NDFap	1.05 a	1.12 a	0.83 b	0.76 b	0.04	<.01
Total digestible nutrients	1.55 a	1.67 a	1.18 b	1.44 a	0.06	<.01
iNDF	0.38 b	0.39 b	0.50 a	0.36 b	0.02	<.01
Milk yield corrected for 3.5 fat, kg/day	16.71	16.64	15.69	14.71	1.35	.69

[a]NDFap, Neutral detergent fibre corrected for ash and protein; iNDF, indigestible neutral detergent fibre; BW, Body weight.
[b]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis). Means followed in the lines by the same letter do not differ by Scott–Knott's test ($p < .05$).
[c]SEM, standard error of the mean.
[d]Probability.

Table 4. Milk composition and performance of F1 Holstein/Zebu cows fed diets with different roughages associated with pseudostem hay of banana trees.

Item (%)[a]	Diets[b] SS	SSPS	EGPS	SCPS	SEM[c]	p Value[d]
Fat	4.23	4.33	4.39	4.15	0.17	.74
Protein	3.55	3.48	3.34	3.55	0.13	.67
Lactose	4.63	4.62	4.63	4.63	0.05	.99
Total solids	13.37	13.43	13.35	13.25	0.27	.96
DDE	9.14	9.1	9.02	9.17	0.17	.93
MUN (mg dL^{-1})	8.53 b	10.58 b	13.22 a	10.27 b	0.74	<.01
Casein	2.76	2.76	2.63	2.83	0.12	.66
Casein/Protein	78.44	79.05	78.49	79.68	0.58	.42
SCC (mil mL^{-1})	103.5	90.12	48.37	97.00	20.04	.38

[a]DDE, Defatted dry extract content; MUN, Milk urea nitrogen; casein/protein – % of casein in relation to protein; SCC, Somatic cell count.
[b]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis). Means followed in the lines by the same letter do not differ by Scott–Knott's test ($p < .05$).
[c]SEM, standard error of the mean.
[d]Probability.

μ is a constant associated with all observations; $T_{k(ij)}$ is the treatment effect "k", with "k" = 1, 2, 3 and 4; Pi is the effect of period "i", with $i = 1$, 2, 3 and 4; A_j is the animal effect "j", with $j = 1$, 2, 3, 4, 5, 6, 7 and 8; IW is the initial body weight as a covariable; and $e_{k(ij)}$ is the experimental error associated with all observations ($Y_{k(ij)}$), which is independent and by hypothesis has a normal distribution with a mean of zero and variance δ_2. Treatments ($T_{k(ij)}$) were considered fixed effects; the animals (A_j), experimental period (P_i), initial body weight and the error term (e_{ijk}) were random effects. When determined as being significant by the F-test, the means of treatments were compared by Scott–Knott's test. Mean values were considered different when $\alpha < 0.05$. When significant, the preference ranking data of cheese samples were subjected to analysis of least significant differences, and it is considered $\alpha = 0.05$. The means for sensory analysis of cheese were analysed by minimum significant difference using the Friedman non-parametric test using the Newell and MacFarlane table.

Results

Diets based on sorghum silage (SS) and sorghum silage with pseudostem hay of banana trees (SSPS) allowed higher dry matter intake (DMI; $p < .01$) and NDFap intake ($p < .01$). Total digestible nutrient intake (TDN, % BW) was lower in elephant grass associated with pseudostem hay of banana trees (EGPS) diet. The crude protein intake was higher with the SSPS diet, while for non-fibrous carbohydrates the highest intake was in the sugar cane with pseudostem hay of banana trees (SCPS) diet, however, the milk yield corrected for 3.5% fat (15.94 kg/day) was similar between diets ($p = .69$; Table 3).

The association of different roughages with banana pseudostem hay in the diet of F1 Holstein x Zebu cows did not influence the chemical composition of milk in relation to fat, protein, lactose, total solids, defatted dry extract, casein and somatic cell count of milk ($p > .05$). The cows fed EGPS had higher values of milk urea nitrogen ($p < .01$; Table 4).

Table 5. Fatty acid profile of milk fat of F1 Holstein/Zebu cows fed diets with different roughages associated with pseudostem hay of banana trees.

	Diets[b]					
Fatty acid[a]	SS	SSPS	EGPS	SCPS	SEM[c]	*p* Value[d]
ΣSFA, mg/g	769.2 a	786.7 a	728.4 b	790.0 a	12.6	<.01
C4:0	2.88	2.76	2.70	2.81	0.09	.60
C6:0	2.15 a	2.19 a	1.99 b	2.22 a	0.05	.03
C8:0	1.43	1.37	1.44	1.48	0.06	.69
C10:0	3.54 a	3.39 a	3.17 b	3.91 a	0.18	.06
C11:0	0.062 b	0.065 b	0.048 b	0.15 a	0.01	<.01
C12:0	4.49 b	4.31 b	3.96 b	5.30 a	0.27	.01
C13:0 iso	0.038	0.045	0.053	0.043	0.004	0.18
C13:0 anteiso	0.11 b	0.11 b	0.11 b	0.16 a	0.008	<.01
C13:0	0.12 b	0.12 b	0.11 b	0.23 a	0.01	<.01
C14:0 iso	0.14	0.27	0.2	0.12	0.05	.20
C14:0	13.41	13.19	12.6	13.75	0.40	.25
C15:0 iso	0.30 b	0.32 b	0.40 a	0.27 b	0.02	.03
C15:0 anteiso	0.53 b	0.51 b	0.69 a	0.42 b	0.05	.02
C15:0	1.23 b	1.28 b	1.32 b	1.85 a	0.11	<.01
C16:0 iso	0.21 b	0.27 a	0.33 a	0.17 b	0.02	<.01
C16:0	37.68	40.21	35.26	40.79	1.62	.09
C17:0 iso	0.28 b	0.29 b	0.43 a	0.25 b	0.02	<.01
C17:0	0.59	0.59	0.63	0.56	0.02	.24
C18:0	7.36 a	7.00 a	7.08 a	4.21 b	0.41	<.01
C20:0	0.14 a	0.14 a	0.11 b	0.07 c	0.007	<.01
C21:0	0.013 b	0.022 a	0.023 a	0.005 b	0.003	<.01
C22:0	0.051	0.060	0.045	0.031	0.006	.03
C23:0	0.032	0.038	0.036	0.021	0.003	.02
C24:0	0.053 a	0.056 a	0.041 b	0.026 c	0.002	<.01
ΣMUFA, mg/g	207.9 b	192.2 b	249.9 a	185.7 b	19.1	<.01
C10:1	0.41 b	0.39 b	0.38 b	0.48 a	0.01	<.01
C12:1	0.13 b	0.13 b	0.12 b	0.19 a	0.01	.71
C14:1c9	1.42 b	1.40 b	1.48 b	1.83 a	0.09	.01
C16:1c9	2.03 b	2.02 b	2.48 a	2.53 a	0.11	<.01
C17:1	0.23 b	0.22 b	0.31 a	0.27 a	0.01	<.01
C18:1 trans11	1.28 b	1.09 c	1.45 a	0.79 b	0.04	<.01
C18:1 c9	14.47 b	13.16 b	17.59 a	11.69 b	1.04	<.01
C18:1 c11	0.53 b	0.52 b	0.78 a	0.51 b	0.05	<.01
C18:1 c12	0.11	0.10	0.12	0.09	0.01	.30
C18:1 c13	0.05 b	0.04 b	0.08 a	0.05 b	0.01	.02
C18:1 t16	0.06 b	0.07 b	0.08 a	0.04 b	0.006	<.01
C18:1 c15	0.006	0.008	0.020	0.016	0.005	.21
C20:1	0.021	0.021	0.026	0.020	0.004	.74
C22: 1n9	0.0	0.0	0.007	0.0	0.003	.25
C24:1	0.022	0.012	0.020	0.016	0.003	.28
ΣPUFA, mg/g	17.2	17.3	17.7	18.9	01.2	.77
C18:3 n6	0.013	0.011	0.015	0.020	0.002	.11
C18:3 n3	0.33 a	0.28 a	0.23 b	0.18 b	0.020	<.01
C18:2 c9t11	0.28 b	0.26 b	0.39 a	0.13 c	0.03	<.01
C18:2 n6	0.90 b	0.98 b	0.90 b	1.29 a	0.08	.01
C20:2	0.006	0.008	0.008	0.008	0.002	.84
C20:3 n6	0.043	0.048	0.051	0.068	0.007	.14
C20:4 n6	0.078 b	0.063 b	0.085 b	0.111 a	0.0090	.01
C20:5 n3	0.021	0.023	0.022	0.017	0.002	.31
C22:5	0.046	0.043	0.050	0.052	0.004	.43
C22:6 n3	0.001	0.001	0.003	0.003	0.001	.45

[a]SFA, Saturated fatty acids; 2 MUFA, monounsaturated fatty acids; 3 PUFA, Polyunsaturated fatty acids.
[b]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis). Means followed in the lines by the same letter do not differ by Scott–Knott's test ($p < .05$).
[c]SEM, standard error of the mean.
[d]Probability.

The effect of diets on milk fatty acid profile was verified in the sum of saturated fatty acids (ΣSFA), being the lowest value with the EGPS diet (Table 5). There was no effect of diets on the concentration of saturated fatty acids C4:0, C8:0, C13:0 iso, C14:0 iso, C14:0, C16:0, C17:0, C22:0 and C23:0 of milk. The C6:0 and C10:0 milk fatty acid profile of animals feed EGPS were lower than the other diets.

For the fatty acid concentrations C11:0, C12:0, C13:0 anteiso, C13:0 and C15:0 the SCPS diet resulted in higher levels. The EGPS diet resulted in higher fatty acid concentrations C15:0 iso, C15:0 anteiso and C17:0

Table 6. Nutritional quality indexes of milk lipid fraction of F1 Holstein/Zebu cows fed diets with different roughages associated with pseudostem hay of banana trees.

	Diets[b]					
Index[a]	SS	SSPS	EGPS	SCPS	SEM[c]	p Value[d]
AI	4.53 a	5.18 a	3.63 b	5.41 a	0.37	.01
TI	2.35 b	2.80 a	2.04 b	2.93 a	0.22	.03
h/H	0.44 b	0.40 b	0.56 a	0.38 b	0.03	<.01
DFA	29.89 a	27.95 b	33.86 a	24.74 b	1.36	<.01
PFA/SFA	0.021	0.021	0.025	0.025	0.002	.44
ω6	0.13 b	0.12 b	0.15 b	0.19 a	0.01	.01
ω3	0.35 a	0.30 a	0.26 b	0.21 b	0.02	<.01
ω6/ ω3	0.37 c	0.40 c	0.59 b	0.93 a	0.04	<.01

[a]AI, Atherogenic index; TI, Thrombogenic index; h/H, hypo/hypercholesterolemic ratio; DFA, desirable fatty acids; PFA/SFA, ratio of polyunsaturated fatty acids/saturated fatty acids; ω6 and ω3 – sum of fatty acids; ω6/ω3 – ratio.
[b]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis). Means followed in the lines by the same letter do not differ by Scott–Knott's test ($p < .05$).
[c]SEM, standard error of the mean.
[d]Probability.

iso. The concentrations of C16:0 iso and C21:0 were higher with SSPS and EGPS diets. Stearic fatty acid (C18:0) presented lower concentration in milk of SCPS fed cows, differing from the others that were higher and similar to each other. For fatty acids C20:0 and C24:0, higher values were observed in milk when the animals were fed the diets SSPS and SS. The sum of monounsaturated fatty acids was higher in the milk of cows fed the EGPS diet, with higher levels of C18:1 trans11, C18:1 c9, C18:1 c11, C18:c13 and C18:t16. The concentrations of the fatty acids C18:1 c12, C18:1 c15, C20:1, C22: 1n9 and C24:1 were not influenced by the diets. In the SCPS diet, C10:1, C12:1 and C14:1c9 fatty acid contents were higher. The C16:1c9 and C17:1 fatty acids presented higher values with the SCPS and EGPS diets. The sum of the polyunsaturated fatty acids was similar between the experimental diets, and the same was true for the fatty acids C18:3n6, C20:2, C20:3n6, C20:5n3, C22:5 and C22:6n3. The C18:3n3 (Linolenic) was higher with SSPS and SS diets. For the milk of cows fed with EGPS, C18:2 c9t11 (CLA) presented higher concentration in relation to the other diets. Moreover, the C18:2c9c12 and C20:4n6 fatty acids presented higher concentrations with the SCPS diet.

Diets with different roughage fractions influenced the indices of evaluation of the nutritional quality of the milk lipid fraction (Table 6). For the atherogenic index (AI), the EGPS diet resulted in lower value. For the thrombogenic index (TI), the lowest values were found in the milk of cows fed with EGPS and SS. Higher values of hypo/hypercholesterolemic fatty acids were observed in the milk of cows fed with EGPS. The concentration of DFA was higher in the diet with SS and EGPs. The PFA/SFA ratio was not influence by the diets; however, the SCPS diet implied a higher value in the sum of ω6 fatty acids and in the ω6/ω3 ratio in milk, when compared to the others. For the sum of ω3 fatty acids, SS and SSPS resulted in higher values.

The chemical composition of the cheese was not influenced by diets with and without banana pseudostem hay associated with different roughages. The yield for cheese moisture was not modified according to the diets (mean 3.49 L/kg; Table 7). For every kilogram of cheese was used 3:49 L of milk. In the acceptance test of Minas Frescal cheese samples by the tasters, only the cheese consistency differed in function of the diets, with lower grade for the EGPS diet. The appearance, flavour and overall impression of the cheeses were not influenced. In the preference test of cheese by tasters, the least preferred cheese was that derived from the milk of cows fed with EGSP diet.

Discussion

Animals feed intake

The higher DMI and NDFap intake by cows with diets based on sorghum silage is explained by the higher dry matter and NDFap content in these diets (Table 1). The highest NFC intake with the SCPS diet is due to the higher proportion of NFC in sugarcane (i.e., sucrose). The higher iNDF concentration (227.4 g/kg) in the EGPS diet implies less fibrous fraction degradability, which limited the TDN intake in this diet, indicating lower energy availability for rumen fermentation (Borges et al. 2019). However, Monção et al. (2020) and Santana et al. (2019, 2020) recommended DMI for F1 Holstein/Zebu cows in the order of 2.50% BW. In this study, although lower DMI was found in cows that received the EGSP and SCPS diet, the DMI is above 2.50% BW not impairing the performance of the animals as justified by milk yield that did not change between treatments.

Table 7. Chemical composition, acceptance test and ranking preference of Minas Frescal cheese of F1 Holstein/Zebu cows fed diets with different roughages associated with pseudostem hay of banana trees.

	Diets[a] Chemical composition[b]					
Item	SS	SSPS	EGPS	SCPS	SEM[c]	*p* Value[d]
Fat (% TDE)	40.58	41.90	40.58	39.05	1.51	.63
Protein (% TDE)	48.18	46.87	48.77	49.28	1.10	.50
Fixed mineral waste (% TDE)	11.22	11.21	11.13	11.66	0.66	.94
TDE (%)	32.28	33.60	33.68	34.64	1.42	.72
Moisture (%)	48.08	45.99	47.72	52.77	2.04	.21
Cheese yield (kg/kg)	4.63	3.53	3.40	3.41	0.09	.32
pH	6.53	6.58	6.59	6.56	0.02	.43
Water activity	0.98	0.98	0.99	0.98	<0.01	.75
		Acceptance test[e]				
Appearance	7.69	7.63	7.52	7.47	–	–
Consistency	7.27 a	7.46 a	6.86 b	7.14a	–	–
Flavour	7.27	7.4	7.15	7.36	–	–
Overall impression	7.38	7.47	7.15	7.38	–	–
Number of tasters	100	100	100	100	–	–
		Ranking preference[f]				
Sum of orders[2]	185a	187a	179b	183a	–	–
Number of tasters	100	100	100	100	–	–

[a]SS, Sorghum silage; SSPS, sorghum silage (70%) associated with 30% pseudostem hay of banana trees (dry matter basis); EGPS – 70% elephant grass (*Pennisetum purpureum* cv. Roxo) with 30% pseudostem hay of banana trees (dry matter basis) and SCPS – 70% sugar cane with 30% pseudostem hay of banana trees (dry matter basis).
[b]TDE, total dry extract; means followed in the lines by the same letter do not differ by Scott–Knot's test ($p < .05$)
[c]SEM, standard error of the mean.
[d]Probability.
[e]Means followed in the lines by the same letter do not differ by minimum significant difference (Friedman's test).
[f]Sum of the orders obtained from the testers to each of the samples. Means followed in the lines by the same letter do not differ by minimum significant difference (Friedman's test)

Milk composition

The milk components have a high relationship with the nutrients ingested from the diets supplied to dairy cows and consequently from the rumen fermentation process. The similarity of milk fat and protein content between experimental diets can be explained by the similarity in their nutritional composition, associated with the observed milk yield level. The results verified comply with current legislation, with values above the minimum limits required (BRASIL 2018). The high roughage: concentrate ratio (75:25) of the evaluated diets also helps to explain the high milk fat content, which is associated with higher rumen acetate concentration and the fact that crossbred cows tend to produce higher fat content in milk (Pimentel et al. 2017; Santiago et al. 2019; Silva et al. 2019).

In the nutrition of ruminants, especially dairy cows, the correct balance of the diet and its nutrients, especially the synchronisation between protein and carbohydrate levels is of great importance. In this context, milk urea nitrogen (MUN) may be an indicator of protein nutrition in lactating cows (Silva et al. 2019). In this work, the higher MUN value found in the EGPS diet may be explained by the higher iNDF concentration (227.4 g/kg) of this diet in relation to the others (155.4 g/kg). Indicating lower ruminal degradability of elephant grass fibrous fraction and, consequently, lower energy availability for microbial synthesis, which can be confirmed by the lower TDN intake with this diet. According to Rosa et al. (2012), the correct balance between roughage, concentrate and energy allows to maintain adequate MUN indexes (10–16 mg dL^{-1}), thus providing increase in milk yield and quality. Although the EGPS diet resulted in the highest MUN value (13.22 mg dL^{-1}), it is within adequate limits for milk, even because casein levels and casein percentage in total milk protein has not changed.

Short-chain fatty acids (SCFAs) of up to 10 carbons, C4:0, C6:0, C8:0 and C10:0 in milk are related to the rumen fermentation process, specifically acetate and beta-hydroxybutyrate. They are precursors for de novo synthesis in the mammary gland of short-chain fatty acids and medium-chain fatty acids in milk (Santiago et al. 2019). The lower concentrations of C6:0 and C10:0 in milk from the EGPS diet may be explained by the lower energy availability of this diet and consequently lower production of precursors for fat synthesis in the mammary gland.

Some of the fatty acids found in milk fat are mainly in saturated form (Eifert et al. 2006), which are associated with cardiovascular disease (Santos et al. 2013). However, the effects associated with increased cardiovascular disease are related to lauric (C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids, while other saturated fatty acids have neutral or positive effects on human health (Mensink et al. 2003). The higher value of lauric acid in milk fat with the SCPS diet may be related to the differences in the carbohydrate profile of this diet with higher proportion of CFN, especially of soluble carbohydrates in relation to the others, which may promote changes in rumen fermentation.

The lower concentration of stearic fatty acid (C18:0) with the SCPS diet may be related to the lower proportions of fibre (392.5 g NDFap/kg) and lignin (99.3 g/kg) found in this diet which may favour a higher rate of fat. passage and shorter time for the action of bacteria in the ruminal biohydrogenation process until the formation of stearic acid (Nudda et al. 2014). Although stearic acid (C18: 0) is saturated, its effect is neutral and has less implication in the lipid profile, because in the body it can be converted to oleic acid that has anti-cholesterolemic effect (Perez et al. 2002).

Monounsaturated fatty acids from milk may come from the diet, from intermediate routes in the rumen biohydrogenation process of polyunsaturated fatty acids or from the action of the enzyme Δ^9 desaturase in stearic fatty acid (C18:0) in the mammary gland (Shingfield et al. 2010). This process may explain the higher concentrations for C14:1c9, C16:1c9, C18:1 *trans* 11 (vaccenic acid) and C18:1c9 (oleic acid) monounsaturated fatty acids, mainly in the EGPS diet that presented the highest concentration of iNDF (227.4 g/kg), and possibly lower ruminal passage rate.

Among the monounsaturated acids, the predominant fatty acid in milk was oleic (C18:1 C9), which in turn is important from a nutritional point of view, since oleic acid is attributed to anticholesterolemic effects (Tsimikas et al. 1999). The increase of vaccinic acid content contributes to the improvement of milk nutritional value, as it is the main precursor of *cis*-9 *trans*-11 CLA in ruminant mammary gland. The increase in vaccenic acid results from the rumen biohydrogenation of polyunsaturated fatty acids, mainly linoleic acid. The increase in CLA with the EGPS diet may be related to the higher concentration of vaccinic acid. CLA found in milk has the C18:2 *cis*-9 *trans*-11 configuration, with C18:1 *trans*-11 (vaccinic acid) as its main intermediate isomer detected (Bauman and Griinari 2001). Increasing it improves the nutraceutical properties of milk. The CLA present in milk fat comes partly from the ruminal biohydrogenation of linoleic acid and partly from the activity of the enzyme Δ^{-9} desaturase in mammary gland cells that transform the absorbed vaccic acid into CLA (Bauman and Griinari 2001).

The similarity in the sum of polyunsaturated fatty acids of milk among the experimental diets is related to low levels ether extract thereof (15.23–21.80 g/kg; Table 1). Lanier and Corl (2015) mention that polyunsaturated fatty acids in milk fat are derived from blood plasma fatty acids, which are derived from fatty acids free from body fat mobilisation and from dietary fatty acids transported as triglycerides by very-low-density lipoproteins (VLDL). Additionally, according to Chilliard et al. (2007), as PUFAs are not synthesised by ruminant tissues, their concentration in milk is determined by the amount of these acids that reach the duodenum. Regarding the nutritional quality of the milk lipid fraction, the atherogenic and thrombogenic indexes show a potential for stimulating platelet aggregation, thus, for lower values of AI and TI, the greater the amount of anti-atherogenic fatty acids found in oils. Thus, the greater the potential for prevention of coronary heart disease (Tonial et al. 2010). For dairy products there are no recommended values for AI and IT, so the lower the values found for these indices, the fatty acid profile becomes more favourable to human health. There are no recommended values for the relationship between hypo and hypercholesterolemic fatty acids (h/H) for dairy products, however, higher values indicate better nutritional quality of lipids found in foods. Higher concentrations of monounsaturated fatty acids, especially oleic acid, CLA and vaccenic acid in the milk of EGPS-fed cows justify the lower AI and higher ratio of hypo/hypercholesterolemic fatty acids, as well as the highest percentage of desirable fatty acids (DFA) in milk.

Cheese quality

Cheese yield depends on milk fat and protein content. Fat content is related to higher water retention in cheese, due to the lower synthesis during cheese elaboration, exerting improvement in the yield. Protein, especially milk casein, has a modified action on the rennet, forming a calcium paracaseinate network that "captures", in different proportions, the other milk elements, such as fat, lactose and mineral salts (Furtado 2005). The results verified in this work for chemical composition and fatty acid profile of milk in function of the evaluated diets justify the values of yield in cheese production.

The Minas Frescal cheese produced from the milk of cows fed diets containing banana pseudostem hay associated with different roughages received 7 points "Moderately Liked", which shows good acceptance by the tasters. The lower grade attributed to cheese from the EGPS treatment for the consistency attribute, besides being the least preferred among the others, is possibly related to the lower concentration of saturated fatty acids in the milk from this treatment, favouring a less firm consistency, since a higher concentration of saturated fatty acids in milk provides greater consistency to cheese (Coppa et al. 2011).

Conclusions

Different roughages associated with pseudostem hay of banana trees in diets of F1 Holstein/Zebu cows did not influence the milk yield corrected for 3.5 fat, chemical composition of milk and cheese. Elephant grass or sugar cane associated with pseudostem hay of banana trees decrease the dry matter intake in F1 Holstein/Zebu cows.

In the lipid fraction of milk, elephant grass diet with banana pseudostem hay favours the increase of total monounsaturated fatty acids, due to the higher concentrations of vaccine and oleic fatty acids in milk, besides increasing the concentration of conjugate linoleic acid, contributing to the improvement of some nutritional evaluation indices of the milk lipid fraction. However, Minas Frescal cheese from the EGPS diet has lower consistency in the acceptance test by the tasters due to the lower concentration of saturated fatty acids in the milk from this treatment.

Ethical approval

The protocol used in this experiment was approved by Montes Claros State University (UNIMONTES; number 166/2018) Animal Care and Use Committee.

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