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Performance and rumen parameters of crossbred dairy cows fed two sugarcane varieties combined or not with soybean hulls

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ABSTRACT - Two sugarcane varieties (IAC86-2480 and IAC91-2195) combined or not with soybean hulls (SH), partially replacing corn in concentrate, were included in the diet of crossbred dairy cows to evaluate the effects on feed intake, milk production, milk composition and rumen parameters of crossbred cows. The sugarcane varieties (IAC86-2480 and IAC91-2195) and concentrate compositions (with and without SH) were analyzed in a 2 × 2 factorial arrangement. Treatments were assigned to blocks by lactation period to evaluate feed intake, milk production and milk composition. A Latin square design was used to analyze rumen parameters. Feed intake, milk production and composition were similar among treatments. Average daily feed intake was 19.93 kg DM in cows fed IAC86-2480 sugarcane and 17.98 kg DM in those fed IAC91-2195, and their average milk production was 19.27 kg and 18.94 kg, respectively. The treatments with IAC86-2480 and IAC91-2195 had different effects on ruminal pH (6.47 and 6.61, respectively) and ammoniacal nitrogen (12.88 and 16.57 mg/dL, respectively). Sugarcane variety and concentrate composition had an interaction effect on volatile fatty acid levels and acetic acid/propionic acid ratio (93.62 mM and 2.54 for IAC86-2480 with SH; 106.70 mM and 2.41 for IAC86-2480 without SH; 115.70 mM and 3.30 for IAC91-2195 with SH; and 93.21 mM and 1.81 for IAC91-2195 without SH, respectively). Sugarcane variety and soybean hull inclusion in feed concentrate do not affect feed intake, milk production or composition in crossbred cows, although these variables change fatty acid production and ruminal pH.

Key Words: by-product, industrial residue, roughage, soybean residue

Introduction

Sugarcane production supplies the ethanol and sugar industries in tropical regions; however, sugarcane can also be used as forage. Cattle growers that use tropical pasture as the main roughage source during the rainy season use sugarcane as roughage supplement in the dry season, when pasture becomes scarce (Oliveira, 1999; Landell et al., 2002).

Sugarcane is composed of a fibrous portion and sugar-rich juice. The fibrous fraction, usually quantified as neutral detergent fiber (NDF), has low digestibility for ruminants. Because juice pol (sucrose) is an energy supplier and NDF limits feed intake in cattle, sugarcane varieties with a NDF/pol ratio lower than 3 are considered suitable as forage (Landell et al., 2002; Santos et al., 2005). The sugarcane variety IAC86-2480 is recommended to be used as forage due to its adequate NDF/pol ratio, long-term usage and resistance against major pests and diseases (Landell et al., 2002). Another sugarcane variety that could be used as cattle feed is IAC91-2195, which has a high sugar content and allows for long-term usage, but to the best of our knowledge, no experiments on this matter have been carried out (Dinardo-Miranda et al., 2008). Regardless of the variety, sugarcane maturity is an important factor to be considered before harvesting, since it must have a minimum brix level (soluble solids) in juice of 180 g/kg; pol between 130 and 150 g/kg; 850 g/kg purity; and a maximum of 10 g/kg of reducing sugars.

According to NRC (2001), soybean hulls (SH) contain approximately 122 g/kg of crude protein (CP), 800 g/kg of total digestible nutrients (TDN) and 663 g/kg of NDF. Its fiber, which is highly digestible for ruminants, can replace corn grain in cattle diets, mainly to decrease costs (Restle et al., 2004).

In the present study, crossbred dairy cattle were fed two sugarcane varieties (IAC 86-2480 and IAC 91-2195), with or without SH as concentrate ingredient, in order to evaluate milk production and quality, feed intake and rumen parameters.

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Material and Methods

Two experiments were carried out to evaluate feed intake, milk production, milk composition and rumen parameters of dairy cows fed diets (Table 1) with different sugarcane varieties and concentrate ingredients (Table 4). Treatments consisted of two sugarcane varieties (IAC86-2480 and IAC91-2195) and concentrate of two different compositions (without soybean hulls (SH) or with SH, instead of 500 g/kg corn in the diet).

The sugarcane varieties were cropped on a slightly wavy Latossolo Vermelho Epidistroférrico (Oxisol) soil (Embrapa, 1999) (Table 2).

Both sugarcane varieties were at harvesting stage according to Brieger (1968), Landell et al. (2002) and Santos et al. (2005). Dry matter (DM) was determined in a forced-air oven.

A chemical analysis of sugarcane varieties (Table 3) was performed to determine neutral detergent fiber free of residual ash (NDF) (Mertens, 2002); crude protein (CP) and acid detergent fiber free of residual ash (ADF) by AOAC (1995) methods; and cellulose and ether extract were determined according to Silva and Queiroz (2002).

Two experiments were conducted to evaluate the milk production (Experiment 1) and ruminal parameters (Experiment 2).

Experiment 1 evaluated DM intake by the crossbred dairy cows by determining total feed intake and feed intake per body weight. It also assessed milk production and quality by measuring total milk production, energy-corrected milk (ECM) and milk composition.

The experiment was conducted in randomized blocks (lactation period), with treatments arranged in a 2 × 2 factorial design consisting of six replications (six cows) per treatment.

Twenty-four crossbred cows (¾ Holstein × ¼ Gir) were milked twice a day (06.00 and 16.00 h) with the calves at foot. The cows underwent a 14-day adaptation period before the 30-day experiment began. Body weight (BW) was measured on a mechanical scale in three phases of the experiment, for three consecutive days in each phase, immediately before and after morning milking. The mean weight obtained in each phase was used to calculate feed intake/BW.

The feed was supplied twice a day, after morning and afternoon milking. Milk production and feed intake were measured daily. Feed samples were taken weekly to determine dry matter, crude protein, NDF and ether extract. Crude protein (CP) was determined using AOAC (1995); NDF analysis was performed to determine neutral detergent fiber free of residual ash (NDF) (Mertens, 2002); and ether extract was determined according to Silva and Queiroz (2002).

Milk composition was evaluated for each cow, which was individually milked twice a week. Milk samples were taken to the laboratory to determine fat, protein, lactose and total solids and for the somatic cell count.

Table 2 - Composition of the soil used for cropping the sugarcane varieties (mean value; per volume of air-dried fine earth)

Sugarcane variety	pH	OM g/dm³	P mg/dm³	K^+	Ca^{2+}	Mg^{2+}	CEC
				mmol/dm³			
IAC86-2480	5.4	34	21.2	4.3	40.9	16.1	97.1
IAC91-2195	5.4	38	24	3.8	49.0	26.0	117

OM - organic matter; P - phosphorus; K^+ - potassium; Ca^{2+} - calcium; Mg^{2+} - magnesium; CEC - cation-exchange capacity.

Table 1 - Proportion of ingredients and chemical composition of the experimental diets containing the different sugarcane varieties (IAC86-2480 and IAC91-2195), with or without soybean hulls (SH) in concentrate

	Experimental diets			
	IAC86-2480		IAC91-2195	
	With SH	Without SH	With SH	Without SH
Ingredients				
Sugarcane (g/kg DM)	600	600	600	600
Cottonseed (g/kg DM)	97	97	97	97
Soybean hulls (g/kg DM)	71	-	71	-
Corn (g/kg DM)	71	143	71	143
Soybean meal (g/kg DM)	143	143	143	143
Urea (g/kg DM)	9	9	9	9
Ammonium sulphate (g/kg DM)	1	1	1	1
Minerals (g/kg DM)	8	8	8	8
Chemical composition				
Dry matter (g/kg)	531	531	519	518
Crude protein (g/kg DM)	146	145	147	146
Ether extract (g/kg DM)	38	40	39	42
Neutral detergent fiber (g/kg DM)	491	451	497	458

Table 3 - Characteristics and chemical composition of the sugarcane varieties

Variety	Juice Brix (g/kg)	Juice Pol (g/kg)	Sugarcane Pol (g/kg)	NDF/Pol ratio	Juice purity (g/kg)	DM (g/kg)	CP (g/kg DM)	Ash (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	Cellulose (g/kg DM)	Lignin (g/kg DM)
IAC86-2480	209	176	162	3.00	919.6	293	19.8	27.4	484	339	256	47.3
IAC91-2195	195	168	156	3.82	926.8	272	21.4	19.4	595	316	275	67.7

Brix - total sugar; Pol - total saccharose; DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber free of residual ash.

Table 4 - Chemical composition of concentrate ingredients

	DM	CP	EE	Ash
Cottonseed (g/kg DM)	871	238	183	27
Soybean hulls (g/kg DM)	891	121	21	48
Corn (g/kg DM)	876	104	48	32
Soybean meal (g/kg DM)	901	496	12	49

DM - dry matter; CP - crude protein; EE - ether extract.

The production of energy corrected milk (ECM) – the amount of energy in the milk based upon milk, fat and protein and adjusted to 35 g/kg fat and 32 g/kg protein – was calculated using the formula: ECM (kg) = (0.327 × milk kg) + (12.95 × fat kg) + (7.65 × protein kg).

Statistical analysis was performed using PROC GLM (Statistical Analysis System, version 9.2) considering repeated measures. The analysis model includes the effects of sugarcane variety, soybean hulls, block, and the interactions between these effects. The interaction between sugarcane variety, soybean hulls and block (residue a) was used to test the hypotheses of the effects of the sugarcane variety, soybean hulls and the interaction between sugarcane variety and soybean hulls. The analysis model also contained the effect of the day of data collection and the interactions between the effects of the sugarcane variety, soybean hulls and day of data collection.

Experiment 2 evaluated the rumen parameters pH, ammoniacal nitrogen ($N\text{-}NH_3$), acetic acid, propionic acid, butyric acid and total volatile fatty acids (VFA).

Rumen parameters were assessed in four rumen-fistulated lactating cows. Milking and feeding were performed as described in Experiment 1. The experiment was arranged in a 4 × 4 Latin square design. Samples of the rumen fluid were collected from the fistula for two consecutive days, before feeding (time 0) and 2, 3, 4, 6, and 9 h post feeding. After nearly 50 mL fluid were collected and filtered, pH was immediately measured with a digital pH meter. A 2-mL aliquot of rumen fluid received 1 mL 1:1 H_2SO_4 solution and was stored at −15 ºC for further $N\text{-}NH_3$ determination. For VFA determination, 1 mL formaldehyde was added to 2 mL fluid and stored at −15 ºC.

Statistical analyses were carried out using PROC GLM (Statistical Analysis System, version 9.2). The analysis model includes the effects of sugarcane variety, soybean hulls, Latin square, and the interactions between these effects. The interaction between sugarcane variety, soybean hulls and block (residue a) was used to test the hypotheses of the effects of the sugarcane variety, soybean hulls and the interaction between sugarcane variety and soybean hulls. The analysis model also contained the effect of the hour of data collection (as a repeated measure) and the interactions between the effects of the sugarcane variety, soybean hulls and hour of data collection.

The statistical model used was:

$$Y_{ijkl} = \mu + B_i + S_j + SH_k + I_{jk} + D_l + e_{ijkl},$$

in which: Y_{ijkl} were the variables of milk quality and production or ruminal parameters; μ was the mean; B_i was the block effect on experiment 1 and Latin square effect on experiment 2; S_j was the fixed effect of sugarcane; SH_k was the fixed effect of soybean hulls; I_{jk} was the interaction between sugarcane and soybean hulls; D_l was the effect of the day of data collection; e_{ijkl} was the random error associated with each observation ijkl, assumed independent and distributed normally with zero mean and variance $\sigma^2 e$.

Results

Feed intake, feed intake/BW, milk production and milk composition were similar between treatments and no interaction effect of sugarcane variety and SH use was found for these parameters (Table 5). Somatic cell score was also similar among treatments (Table 5).

Ruminal pH and ammoniacal N levels were higher in cows fed sugarcane variety IAC91-2195 (Table 5 and Figure 1), but propionic acid levels in rumen fluid were not affected by sugarcane variety (Table 5 and Figure 1). Concentrate composition did not affect ruminal pH and ammoniacal N levels, but propionic acid levels were higher in the rumen fluid of cows fed concentrate without SH (Figure 2). No interaction effect of sugarcane variety and SH use was recorded for pH, ammoniacal N and propionic acid levels in rumen fluid.

An interaction effect of sugarcane variety and SH use was found for other rumen parameters (Table 6). Sugarcane

IAC91-2195 combined with SH increased acetic acid, butyric acid and VFA levels as well as the acetic acid/propionic acid ratio in rumen fluid as compared with the other treatments (Table 6; Figure 3).

Discussion

It is unlikely that sugarcane is voluntarily consumed by cattle because of its low crude protein, high lignin content and low digestibility (Magalhães et al., 2006; Mendonça et al., 2004). The dairy cows studied had adequate mean daily feed intake (18.95 kg DM/cow) and feed intake/BW (3.49 kg DM/100 kg BW). Feed intake was close to the 17.26 kg DM cow/day reported in another study of cows with similar milk production potential and receiving a similar roughage:concentrate ratio (Magalhães et al., 2006).

Feed intake was maintained at the same volume irrespective of SH inclusion in the diet (Table 5). Mendes et al. (2005) studied the partial substitution of corn for SH in diets for steers. The partial substitution did not affect the intake of dry matter from the feed and the weight gain of animals, and the authors concluded that if economic analysis is favorable, the energy source can be replaced by SH.

Prohmann et al. (2004) also studied the weight gain of steers growing on coast-cross grass during summer. Four treatments were conducted using diets with the following levels of SH: 0, 0.2, 0.4, and 0.6% of live weight (DM basis). The author concluded that different levels of SH did not affect the weight gain.

In the present study the inclusion of SH in the diet or the sugarcane varieties did not affect milk production and composition (Table 5). Mendonça et al. (2004) and Magalhães et al. (2004) worked with substitution of corn silage for sugarcane, with a similar roughage:concentrate ratio of 60:40. Magalhães et al. (2004) observed milk production of 20.36 kg/cow/day and Mendonça et al. (2004) found 18.6 kg/cow/day, which is close to results of the present experiment.

The average fat content in the milk was low (29.3 g/kg; Table 5), probably because of the low degradation of the sugarcane fiber. This indicates that the increase

Table 5 - Milk production and composition and rumen parameters in crossbred dairy cows fed two sugarcane varieties combined or not with soybean hulls[1]

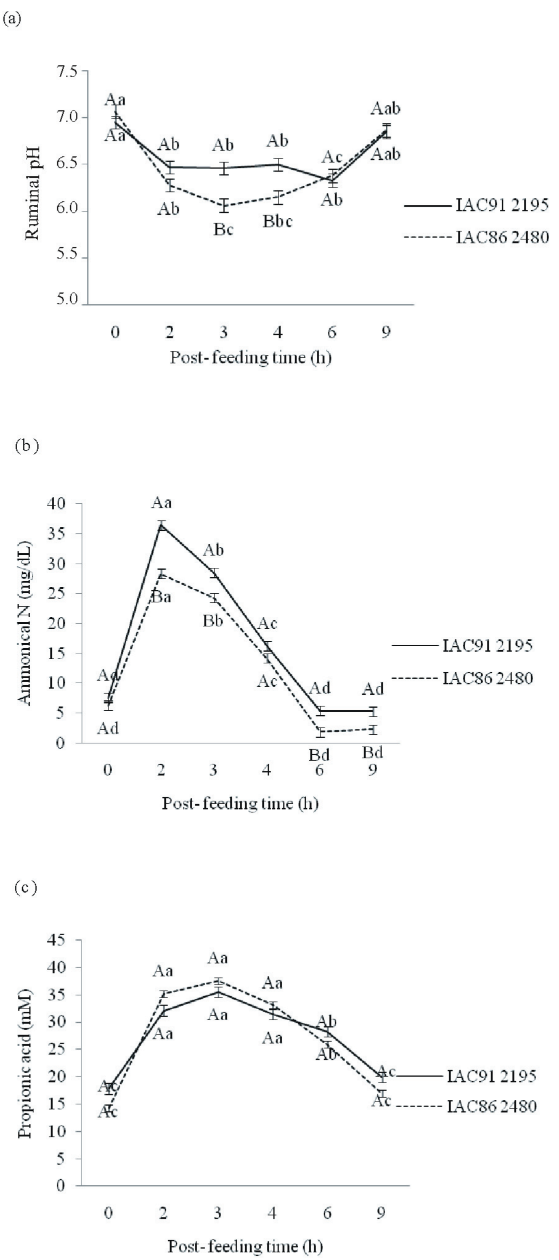
Variable	Sugarcane variety		P-value	Soybean hulls		P-value	P interaction
	IAC86-2480	IAC91-2195		With	Without		
Productivity							
DM intake (kg/day)	19.93 (1.87)	17.98 (1.35)	0.5067	19.00 (2.09)	18.90 (1.58)	0.9251	0.5703
DM intake (g/kg BW)	36.8 (0.35)	32.9 (0.25)	0.4940	35.0 (0.39)	34.7 (0.29)	0.9094	0.5493
Milk yield (kg/day)	19.27 (0.99)	18.94 (1.11)	0.8511	19.32 (0.87)	18.89 (1.21)	0.5990	0.9412
ECM (kg/day) production	18.25 (1.17)	17.98 (1.31)	0.8527	18.45 (1.03)	17.82 (1.43)	0.4759	0.8548
Milk composition							
Fat (g/kg)	29.1 (0.17)	29.5 (0.21)	0.7939	29.8 (0.20)	28.9 (0.18)	0.5860	0.6145
Protein (g/kg)	31.8 (0.06)	31.4 (0.07)	0.5434	31.6 (0.07)	31.6 (0.06)	0.9039	0.8489
Lactose (g/kg)	44.8 (0.07)	44.6 (0.08)	0.6298	44.4 (0.08)	45.0 (0.07)	0.6903	0.4427
Total solids (g/kg)	115.5 (0.22)	114.9 (0.25)	0.7961	115.6 (0.26)	114.8 (0.22)	0.6097	0.9543
Somatic cell score[2]	5.05 (0.34)	5.48 (0.40)	0.3882	5.66 (0.41)	4.89 (0.33)	0.1142	0.8908
Rumen parameters							
Ruminal pH	6.47b (0.02)	6.61a (0.02)	0.0325	6.55 (0.02)	6.53 (0.02)	0.7666	0.0881
$N\text{-}NH_3$ (mg/dL)	12.88b (0.78)	16.57a (0.79)	0.0252	13.70 (0.79)	15.75 (0.79)	0.1197	0.6887
Propionic acid (mM)	27.19 (0.64)	27.52 (0.64)	0.7857	23.88b (0.64)	30.83a (0.64)	0.0002	0.9695

ECM - energy corrected milk; BW - body weight.
[1] Means followed by the standard errors of the mean (SEM).
[2] Somatic cell score - log somatic cell count.

Table 6 - Rumen parameters in crossbred dairy cows fed two sugarcane varieties combined or not with soybean hulls[1]

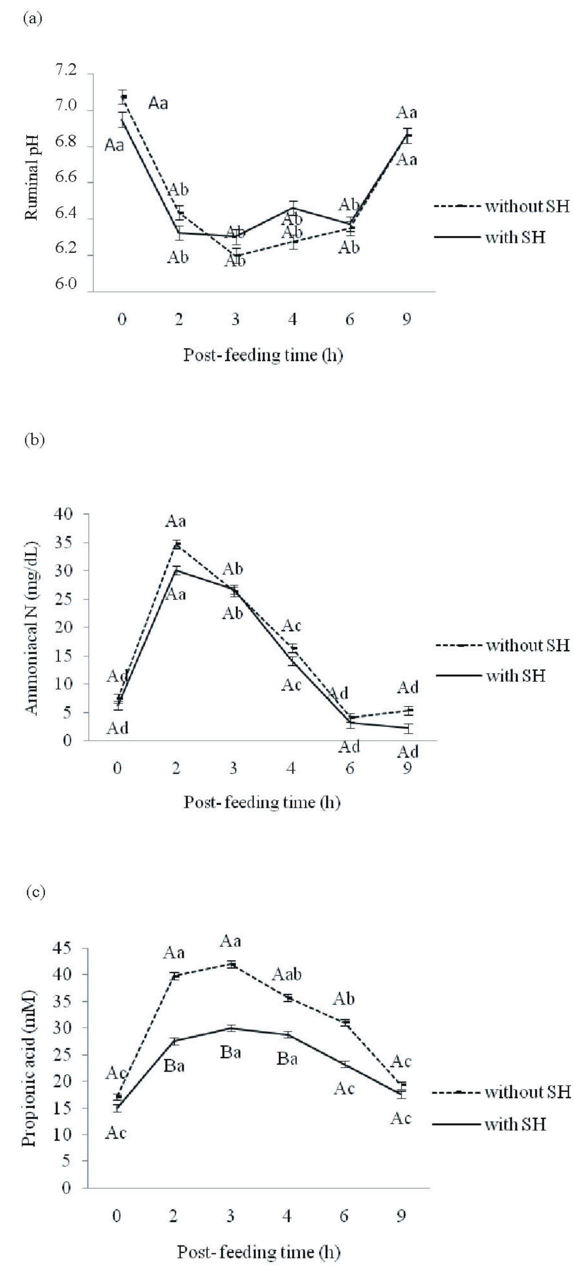
Parameters	IAC86-2480		IAC91-2195		P-value		P interaction	SEM
	With SH	Without SH	With SH	Without SH	Sugarcane	SH		
Acetic acid (mM)	56.40c	62.55b	75.44a	52.64c	0.0460	0.0023	<0.0001	1.58
Butyric acid (mM)	13.53b	13.46b	15.66a	9.60c	0.2984	0.0036	0.0041	0.39
Volatile fatty acids (mM)	93.62c	106.70b	115.7a	93.21c	0.2425	0.2010	0.0004	2.65
Acetic acid/propionic acid ratio	2.54b	2.41b	3.30a	1.81c	0.3609	<0.0001	<0.0001	0.06

SH - soybean hulls.
[1] Means followed the standard errors (SEM).
Different letters in a row indicate a statistical difference between treatments.



Means followed by the same uppercase letter between lines and lowercase letter in a line are statistically equal (P>0.05).

Figure 1 - Influence of sugarcane varieties on ruminal pH (a), ammoniacal N (b) and propionic acid (c).



Means followed by the same uppercase letter between lines and lowercase letter in a line are statistically equal (P>0.05).

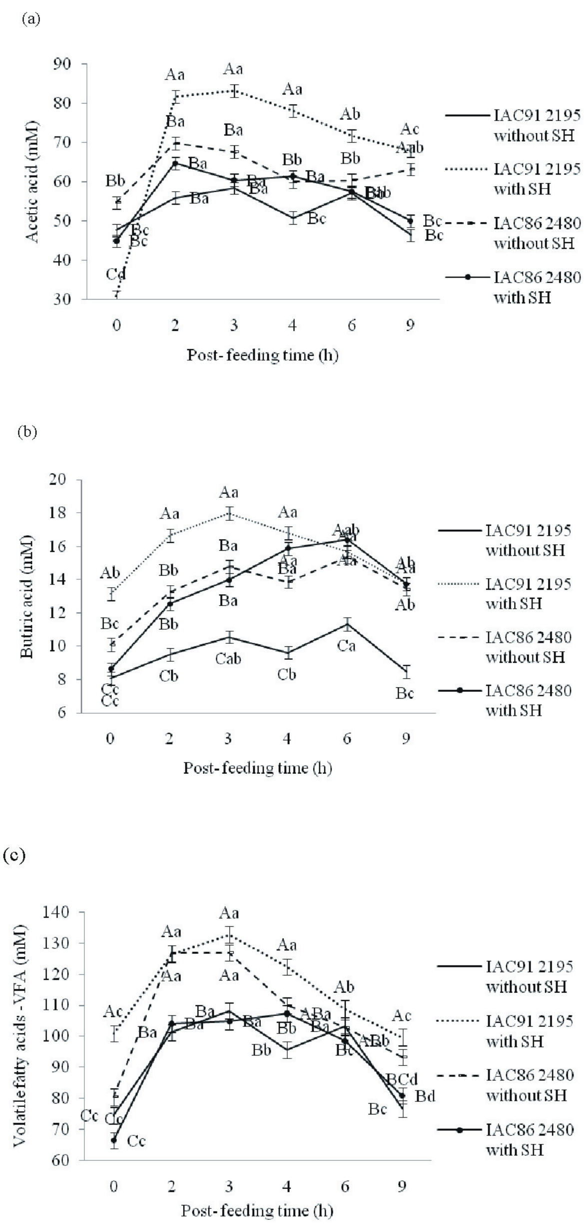
Figure 2 - Influence of soybean hulls (SH) in concentrate on ruminal pH (a), ammoniacal N (b) and propionic acid (c).

in digestible fiber content in concentrate, promoted by replacing 500 g/kg corn grain with SH, was not enough to increase milk fat.

Because of the SH behavior in the rumen, it is classified as a rapidly fermented fiber (Zambom et al., 2001) that can be used as an energy source and to maintain adequate dietary fiber levels in the diet. As a result, the use of SH prevents a decrease in ruminal acetic acid levels and milk fat.

Pantoja et al. (1994) reported that SH used as a fiber source likely does not interfere in the effects promoted by other dietary ingredients such as unsaturated fats, but the SH increased the digestion of NDF and fat content in milk. In the present study, SH as a substitute for 500 g/kg dietary corn did not affect milk composition (Table 5), but raised acetic acid levels in the rumen, especially when combined with the IAC91-2195 variety (Figure 3a).

No pH reduction below 5.7, which inhibits fiber degradation in the rumen (Dijkstra et al., 2012), was observed in the present study. Post-feeding time, diet and animal salivation are factors that directly affect rumen pH (Queiroz et al., 1998; Dijkstra et al., 2012) and the development of ruminal microorganisms. In addition, protein synthesis by the ruminal microbiota is also affected by the metabolism of nitrogen compounds, as indicated by ruminal ammonia levels (Wallace et al., 1997). Dijkstra et al.



Means followed by the same uppercase letter between lines and lowercase letter in a line are statistically equal (P>0.05).

Figure 3 - Acetic acid (a), butyric acid (b) and total volatile fatty acids (VFA) (c) in the rumen of cows fed diets containing two sugarcane varieties and concentrate with or without soybean hulls (SH).

(2012) reported the presence of VFA in the rumen 2 to 4 h post feeding, when pH is between 5.8 and 6.6, and stated that higher or lower pH values affect VFA production. Accordingly, the VFA peaks detected in the present study were between 2 and 4 h post feeding, when the rumen pH ranged from 6.2 to 6.4 (Figures 1 and 2).

In a study on animals fed diets with a 60:40 roughage: concentrate ratio, 450 g/kg NDF and 270 g/kg ADF, Bachman (1992) found that the rumen pH was nearly 6.2, acetic acid 64 mM and propionic acid 22nM, and that these values depended exclusively on food quality and characteristics. The results obtained by that author are similar to those recorded in the present study (Tables 5 and 6), except for a number of rumen parameters that were higher in the treatment combining the IAC91-2195 variety and SH in concentrate (Table 6).

According to Black (1990), the typical molar proportion of VFA (acetic:propionic:butyric acid) is 60:30:10 in the rumen of animals fed diets containing both forage and concentrate. In this study, these proportions were 66:21:13 for the diet consisting of IAC91-2195 with SH; 57:33:10 for IAC91-2195 without SH; 60:25:15 for IAC86-2480 with SH; and 59:29:12 for IAC86-2480 without SH.

Diet factors that stimulate propionic acid production or change the acetate:propionate ratio affect the fat content in milk (Kennelly, 2000). Fat levels can be increased by the high molar mass of acetic acid and butyric acid and lowered by the molar mass of propionic acid. In the present study, the tested diets affected the ratio between acetic acid and propionic acid but not the milk fat content, which was considered low in all the treatments.

The results obtained in the dietary treatments with different sugarcane varieties studied, irrespective of SH inclusion, were within the expected range, as were mean ammonia levels (>5 mg/dL of rumen fluid) in all the treatments (Table 5). Nevertheless, animals fed sugarcane IAC91-2195 exhibited higher ammonia production than those fed IAC86-2480. This increase is likely related to the higher levels of NDF, cellulose and lignin in the IAC91-2195 variety (Table 3).

For the IAC86-2480 variety, ammonia production reached 25.47 and 31.20 mg $N\text{-}NH_3$/dL in treatments with and without HS, respectively. Although a decline in pH was also observed in the rumen fluid of animals 2 h post-feeding, ruminal microbial activity was not compromised. Normal ammonia and pH levels were recovered 4h post-feeding.

According to Van Soest (1994), the optimal rumen ammonia level is 10 mg/100 mL, but this value cannot be fixed because the capacity of bacteria to use ammonia for protein synthesis depends on the carbohydrate fermentation rate. Some studies suggest that the ammonia concentration required to support maximum microbial protein synthesis is from 15 to 20 mg N-NH_3/100 mL of fluid and that it depends on the diet (Leng and Nolan, 1984).

The described rumen parameter values are expected for cows fed sugarcane and roughage-based diets since they have elevated levels of highly soluble carbohydrates, resulting in rapid sugar fermentation and lower pH. Moreover, because sugarcane fibers are difficult to digest, sugarcane likely contributes to increased ammonia levels, since, as reported by Ribeiro et al. (2001), it allows for the detection of an imbalance in protein digestion, that is, high ammonia levels can indicate excessive dietary protein degradation, whereas low ammonia levels may indicate low carbohydrate degradation in the rumen.

Mendonça et al. (2004) evaluated pH and ammonia levels in the rumen fluid of dairy cows fed sugarcane and found that pH declined from 7.2 to 6.8 and N-NH_3 levels increased from 3.8 to 16.6 mg/dL from 0 to 3 h post-feeding. The results found in the present study (Figures 1b and 2b) are closer to those obtained by Magalhães et al. (2006), who evaluated the rumen fluid of dairy cows fed sugarcane. For periods 0 (before feeding) and 2, 4 and 6 h post-feeding they found pH values of 6.87, 6.71, 6.60 and 6.79 and N-NH_3 levels of 4.66, 20.25, 12.56 and 7.81 mg/dL, respectively.

Conclusions

Sugarcane varieties IAC86-2480 and IAC91-2195 in the diet of crossbred dairy cows, with or without soybean hulls in the concentrate, do not affect feed intake, milk production and composition. The diet combining soybean hull in concentrate and the sugarcane variety of higher neutral detergent fiber and lignin contents increases the production of volatile fatty acids in the cow rumen. Replacing corn with soybean hulls in the concentrate fraction of sugarcane-based diets is an alternative solution and the use of this ingredient depends on its availability in the market.

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