Nota / Note

RUMINAL PARAMETERS ANALYZED IN REMAINING DIGESTION RESIDUE OF ROUGHAGES IN THE *IN VITRO*/GAS SYSTEM[1]

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ABSTRACT: Animal performance is the most direct measure in the evaluation of feed quality. However, performance data are often insufficient to determine possible interactions that may take place in the ruminal environment. The purpose of the present trial was to evaluate the possible associative effects on the concentrations of volatile fatty acids (VFAs), ammoniacal nitrogen ($N\text{-}NH_3$) and pH in the remaining liquid fraction from the dry matter (DM) digestion for exclusive roughages: sugarcane (SC), 60- (EG60) and 180-day elephantgrass (EG180), and corn silage (SIL), as well as for combined roughages: sugarcane+corn silage (SCSIL), sugarcane+60-day elephantgrass (SCEG60), sugarcane+180-day elephantgrass (SCEG180), corn silage+60-day elephantgrass (SILEG60), corn silage+180-day elephantgrass (SILEG180) associated at equal DM proportions (50%). These associative effects present positive or negative effects on bovine performance. Concentrations of VFAs and $N\text{-}NH_3$, as well as pH for the treatments were, respectively: SC= 56.9 mmol L^{-1}, 50.1 mg dL^{-1}, 5.7; SCSIL= 61.4 mmol L^{-1}, 50.7 mg dL^{-1}, 5.8; SCEG60= 54.7 mmol L^{-1}, 47.6 mg dL^{-1}, 5.8; SCEG180= 45.4 mmol L^{-1}, 49.4 mg dL^{-1}, 6.0; SIL= 57.2 mmol L^{-1}, 54.0 mg dL^{-1}, 5.8; SILEG60= 57.1 mmol L^{-1}, 53.1 mg dL^{-1}, 5.9; SILEG180= 55.9 mmol L^{-1}, 52.3 mg dL^{-1}, 6.0; EG60= 58.1 mmol L^{-1}, 49.4 mg dL^{-1}, 5.9; and EG180= 44.0 mmol L^{-1}, 46.4 mg dL^{-1}, 6.1. Nonstructural carbohydrates and starch, associated with fiber and protein, contributed to positive associative effect on the 50:50 sugarcane/corn silage mixtures. The high fermentative aspect of such mixture may have promoted the best results in bovine performance.
Key words: VFAs, $N\text{-}NH_3$, elephantgrass, corn silage, sugarcane

PARÂMETROS RUMINAIS ANALISADOS EM RESÍDUOS REMANESCENTES DA DIGESTÃO *IN VITRO*/GÁS DE VOLUMOSOS

RESUMO: O desempenho animal é a medida mais direta de se avaliar a qualidade dos alimentos. Entretanto, dados de desempenho são insuficientes para se detectar as possíveis interações que possam ocorrer no ambiente ruminal. O objetivo do presente trabalho foi avaliar os possíveis efeitos associativos nas concentrações de ácidos graxos voláteis (AGVs), nitrogênio amoniacal ($N\text{-}NH_3$) e pH da fração líquida remanescente da digestão da matéria seca (MS) de volumosos exclusivos (cana-de-açúcar= CN; capim-elefante com 60 dias= CP60 e 180 dias= CP180 de crescimento; e silagem de milho= SIL) e suas combinações (cana-de-açúcar+silagem de milho= CNSIL; cana-de-açúcar+capim-elefante-60d= CNCP60; cana-de-açúcar+capim-elefante-180d= CNCP180; silagem de milho+capim-elefante-60d= SILCP60; silagem de milho+capim-elefante-180d= SILCP180) na proporção de 50% na MS, que levam a resultados de desempenhos positivos ou negativos de bovinos. As concentrações de AGVs, $N\text{-}NH_3$ e pH dos tratamentos foram: CN= 56,9 mmol L^{-1}, 50,1 mg dL^{-1}, 5,7; CNSIL= 61,4 mmol L^{-1}, 50,7 mg dL^{-1}, 5,8; CNCP60= 54,7 mmol L^{-1}, 47,6 mg dL^{-1}, 5,8; CNCP180= 45,4 mmol L^{-1}, 49,4 mg dL^{-1}, 6,0; SIL= 57,2 mmol L^{-1}, 54,0 mg dL^{-1}, 5,8; SILCP60= 57,1 mmol L^{-1}, 53,1 mg dL^{-1}, 5,9; SILCP180= 55,9 mmol L^{-1}, 52,3 mg dL^{-1}, 6,0; CP60= 58,1 mmol L^{-1}, 49,4 mg dL^{-1}, 5,9; CP180= 44,0 mmol L^{-1}, 46,4 mg dL^{-1}, 6,1. Os carboidratos não estruturais e amido, aliados à fibra e proteína, contribuíram para que ocorresse o efeito associativo positivo na mistura 50:50 cana/silagem. Isso pode ter propiciado os melhores resultados de desempenho em bovinos devido ao elevado padrão fermentativo.
Palavras-chave: AGVs, $N\text{-}NH_3$, capim-elefante, silagem de milho, cana-de-açúcar

INTRODUCTION

The availability and quality of pastures during the dry season are the two main factors affecting cattle performance. However, some forage plants have higher tolerance to climate variations than others, without severely affecting production, as it is the case of elephantgrass (*Pennisetum purpureum* Schum) and sugarcane (*Saccharum officinarum* L.), which are widely used as cattle feed.

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Stored roughages are used during the dry season, especially corn silage, which is produced out of excess corn (*Zea mays* L.) production or from corn grown specifically for such use. Nonetheless, production costs can become too high when considering feeding all animal categories. A reasonable alternative would be providing a mixture of roughages in order to minimize, expenses on feed and losses in animal production. The combination of two or more roughages can often reduce diet quality, due to variations in protein and energy contents, as well as in the roughage degradation rates.

Animal performance is the most direct measure when evaluating feed quality. However, performance data are insufficient to determine and explain possible interactions that may occur in the rumen. Most studies on roughage combinations only evaluate animal performance, and only a few analyze cause-effect relationships of roughage combinations as to the energetic or protein metabolite contribution, ruminal fermentation compounds and their possible associative effects leading to positive or negative results in animal performance. Castro et al. (1967) studying the substitution of sugarcane for corn silage at percentages of 25, 50, 75 and 100%, in half-breed cows (Holstein/Zebu), did not find differences in milk production among treatments. As corn silage percentage increased, there was a higher roughage DM consumption. Caielli (1975) evaluated the performance of Gir calves fed elephantgrass, sugarcane or a mixture of elephantgrass:sugarcane (1:1), supplemented with 40% of concentrate. Animals receiving the elephantgrass and elephantgrass:sugarcane mixture showed higher weight gain than those receiving only sugarcane as roughage (0.64, 0.66 and 0.61 kg day^{-1}, respectively).

Biondi et al. (1978) evaluated the partial and total substitution of corn silage for sugarcane (0, 25, 50, 75 and 100% of roughage DM), after correcting the protein amounts of the treatments through the addition of concentrates to the diet. The authors found a linear reduction in milk production when sugarcane was added to the diet and concluded that, when correctly supplemented, sugarcane could substitute corn silage up to 50%. Ferreira et al. (1989) studied the substitution of sugarcane for corn silage in confined calves' at proportions of 100:0, 50:50, 25:75, and 0:100 (DM percentage) for sugarcane and corn silage, respectively. There was an increase in dry matter consumption, as well as in weight gain of animals, as corn silage substitute sugarcane. Ferreira et al. (1991) fed confined Nelore cattle on corn silage, sugarcane or corn silage/sugarcane (1:1) and observed that the treatments corn silage and corn silage/sugarcane gave the best results ain weight gain and feed conversion.

Ruggieri et al. (1996) carried out *in situ* tests on the degradability of dry matter and neutral-detergent fiber (NDF) of sugarcane, corn silage and corn silage/sugarcane (1:1). Corn silage DM degradability was similar to that of sugarcane and corn silage/sugarcane, although NDF degradability of corn silage was higher in relation to the other treatments. As to the sugarcane/corn silage combination, there was an increase in the degradable and potentially degradable fractions of this mixture, with similar results verified for the effective degradability of the mixture in relation to sugarcane.

Pires et al. (1999) evaluated the productive performance and ruminal parameters of lactating Holstein cows, fed on concentrate and roughage at equal dry basis proportions (50%). The authors also studied the effects of substituting corn silage for sugarcane at proportions, on roughage dry basis, of 100:0 (control), 75:25, 50:50, 25:75 and 0:100%, with the concentrate being balanced in order to make diets isoenergetic and with equal amounts of protein. Higher concentrations of acetic, propionic and butyric acids (79.38, 27.27, 14.47 mmol L^{-1}, respectively) were found in the ruminal liquid of animals fed on the 50:50 sugarcane/corn silage mixture, when compared to those receiving corn silage as exclusive roughage (70.41, 23.80, 11.91 mmol L^{-1}, respectively, for the same parameters). Animals receiving the 50:50 sugarcane/corn silage mixture produced more milk (18.08 Kg day^{-1}) than those receiving 100% corn silage (16.50 kg day^{-1}), with similar dry matter consumptions (14.30 and 14.86 kg day^{-1}, respectively).

Feed DM degradation in digestibility tests depends on many factors, such as chemical and physical characteristics of the incubated feed, ruminal environment and also the feed intake capability of the animal. Plant morphology, fiber crystal structure, as well as other factors unrelated to chemical composition, may also affect dry matter degradability of the incubated substrate (Mertens, 1977).

The importance of measuring VFAs in time in *in vivo* digestibility research lies on determining the concentration, variation and the amplitude of the absorption capability of ruminal VFAs. However, in the *in vitro*/gas digestibility process, the VFAs concentrations are evaluated, only at the end of the 48-hour digestion period, providing useful information when the concentrations in several type of feed are relatively compared. According to Sutton (1985), VFAs produced through ruminal substrate fermentation, and subsequently absorbed, represent the greatest energy source, yielding at least 50% of the total digestible energy.

The evaluation of combined roughages degradability is still little used in our conditions. If the contribution of compounds from ruminal fermentation of combined roughages is known, it is possible to properly adjust the diet according to animal category and productive level. The purpose of the present trial was to analyze the concentrations of volatile fatty acids (VFAs), ammonia-nitrogen ($N\text{-}NH_3$) and pH, in the liquid fraction of the remaining digestion residue of exclusive roughages and their combinations, after a 48-hour digestion period using the computerized monitoring system of *in vitro* gas

production, in order to detect possible associative effects of roughage combinations and the causes that impact animal performance.

MATERIAL AND METHODS

Exclusive roughages, as well as combined in pairs at equal DM proportions (50%), used in evaluation of ruminal parameters after a 48-hour digestion period through the *in vitro*/gas method, according to methodology described by Pell & Schofield (1993) and evaluated by Campos et al. (2000), were as follows: 1- Sugarcane – 2nd harvest, RB72454 (SG); 2- Sugarcane + corn silage (SCSIL); 3- Sugarcane + 60-day elephantgrass (SCEG60); 4- Sugarcane + 180-day elephantgrass (SCEG180); 5- Corn silage, XL360, (SIL); 6- Corn silage + 60-day elephantgrass (SILEG60); 7- Corn silage + 180-day elephantgrass (SILEG180); 8- 60-day elephantgrass, CV Taiwan 148, (EG60); 9- 180-day elephantgrass (EG180).

In order to analyze the concentration of volatile fatty acids and ammonia nitrogen, 4 mL of the liquid fraction of the digestion residue were taken from each duplicate incubated sample, and 1.0 mL of metaphosphoric acid at 25% was added. The material was centrifuged for 20 minutes at 4°C, under 12,000 x g. The floating phase was collected and stored in a freezer, in 20-mL polyethylene flasks, for further analyses of volatile fatty acids (VFAs) and ammonia nitrogen ($N\text{-}NH_3$), according to methodology described by Palmquist & Conrad (1971).

The VFA concentrations were determined by means of liquid-gaseous chromatography (LGC). The chromatograph, Hewlett Packard 5890 series II, connected to a Hewlett Packard 3396 integrator, series II, was equipped with a 6-feet aluminum column (183 cm) of ¼ " diameter (6.35 mm) packed in 80-120 A/W carbopack, and a flame ionization detector. The gases used were: carrier gas - nitrogen, at flow level of 20 mL min^{-1}; oxidant agent – oxygen, at flow level of 400 mL min^{-1}; fuel – hydrogen, at flow level of 30 mL min^{-1}. The operating temperatures of the vaporizer, the separation column and the detector were 150° C, 115° C and 190°C, respectively.

VFA concentrations were determined through placing 1.0 mL of the liquid floating phase of the digestion residue in glass flasks with screwing lids, after adding 0.1 mL of solution containing 10 mL alcohol ethanol, 1.1616 g of 2-ethyl-butyric acid and 90 mL of deionized water, and this was used as the internal standard. This solution was used as quantitative peak reference for the analyses. The chromatograph was calibrated with a reference sample by successive readings until the peaks stabilized.

After every ten readings of ruminal liquid samples, the reference samples were used for new calibration. VFA concentrations for the reference sample were 61.62 mmol L^{-1} acetic acid (C_2), 20.82 mmol L^{-1} propionic acid (C_3), 15.78 mmol L^{-1} butyric acid (C_4), 2.27 mmol L^{-1} isobutyric acid (iC_4), 3.07 mmol L^{-1} valeric acid (C_5) and 3.77 mmol L^{-1} isovaleric acid (iC_5). Results for final VFA concentrations were corrected using a 1.25 correction factor, obtained when diluting the metaphosphoric acid added to remaining liquid samples from the *in vitro* digestion.

Ammonia nitrogen concentration (remaining liquid from the *in vitro* digestion) was measured according to the methodology described by Chaney & Marback (1962), adapted for the use of continuous rectangular plates with 96 cells (containers) for subsequent reading on colorimetric equipment – Elisa – model 3550, equipped with a 540-nm lamp. The samples were the same as those collected for VFAs analysis. Aliquots of 40 μL of the ruminal floating liquid (stock solution) were added, to each tube, to 40 μL of deionized water, 2.5 mL of phenol reagent and 5.0 mL of sodium hypochlorite. The tubes were incubated in water bath at 37°C (previously stabilized) for 10 minutes. For plotting the standard curve, 300-μL duplicates were pipetted from each tube, as well as standard solutions at the following dilution levels: 0, 1, 2, 4, 8, 16 and 32 mg dL^{-1}. Absorbance values were converted to mg of $N\text{-}NH_3$ dL^{-1} through the standard curve. The values were corrected through the 1.25 factor obtained through metaphosphoric acid dilution in the ruminal liquid. pH readings were carried out at the end of the digestion of the triplicate incubated samples, individually collected from each incubation flask.

Concentration results for VFAs, $N\text{-}NH_3$ and pH for the *in vitro* dry matter digestion were submitted to analysis of variance by using the SANEST/USP software (Sarriés et al., 1993) and treatment means were compared by Tukey test at 5%. A statistical analysis following a completely randomized design, with nine treatments and three replicates, was carried out for ammoniacal nitrogen ($N\text{-}NH_3$) and hydrogen-ionic potential (pH). As for volatile fatty acids (VFAs), the same statistical design was employed, but with two replicates per treatment.

RESULTS AND DISCUSSION

Acetic acid (C_2) concentrations in the liquid fraction of the digestion residue for individually analyzed roughages were similar (P>0.05), except for treatments EG180, SCEG180, which had lower concentration levels (P<0.05) than the other roughages (Table 1). For such treatments, the low acetic acid concentration may be related to the low fiber nutritional quality, which inhibited microbial growth, thus quantitatively reducing the metabolic products, such as VFAs and gases. This was also evident in the results of DM degradability (Table 1) presented by the remaining digestion residue.

Propionic acid (C_3) concentrations in the liquid digestion residue of corn silage and SCSIL combined roughages tended to be higher, although not different from the other treatments', except for EG180, SCEG60

and SCEG180, which showed lower concentrations. For butyric acid (C_4) there was a tendency for higher concentrations in the SCSIL mixture, although with no difference among treatments (Table 1).

Acetic acid concentration in the SCSIL mixture was higher (37 mmol L^{-1}) ($P>0.05$) than the concentrations found in each roughage individually (sugarcane and corn silage) and higher than the average for both roughages (34.0 mmol L^{-1}), indicating a positive associative effect (Table 1). The same was observed for butyric acid and T-VFAs concentrations, which showed a difference of 4.35 mmol L^{-1}, which is superior than the average results for sugarcane and corn silage (57.05 mmol L^{-1}) (Table 1). These results indicate the qualitative value of the SCSIL mixture, probably due to the associative effect among sugars, starch and proteins, which may explain the higher animal performance (Ferreira et al., 1989; Ferreira et al., 1991; Pires et al., 1999) using the same roughage proportions analyzed in this experiment. Another roughage mixture with promising results in the present trial, as to the DM degradability and VFAs concentration, was SCEG60 (sugarcane/60-day elephantgrass), which could partially substitute 60-day elephantgrass qualitatively without the animals undergoing energy deficit, once adapted to this new diet.

VFA concentrations found in this trial were similar to those obtained by Stefanon et al. (1996), who evaluated the VFAs concentrations in the liquid fraction of the *in vitro*/gas digestion residue for alfalfa (*Medicago sativa* L.) at five different maturity stages. The authors verified a decrease in concentration as maturity progressed, with the following values found: 58.2 to 37.9, 37.0 to 24.7, 18.3 to 11.4 and 2.9 to 1.8 mmol L^{-1} for total VFAs, acetic, propionic and butyric acids, respectively. Doane et al. (1997), evaluating the *in vitro*/gas digestibility for bromegrass (*Bromus inermis* L.) at two maturity stages, verified that VFA production after a 48-hour digestion period were 36.3 and 38.4, 23.0 and 19.7, 6.1 and 4.8, 65.4 and 62.8 mmol L^{-1} for acetic, propionic and butyric acids, as well as for total VFAs, respectively. Such results were similar to those found in the present experiment, with a few differences probably due to the use of distinct roughages.

Valvasori et al. (1998) evaluated alterations in ruminal fermentation of cattle fed sugarcane as a substitute for corn silage (0, 1/3, 2/3 and 1) and found the following VFA concentration for sugarcane diet: 93.9, 56.3, 22.7 and 15.0 mmol L^{-1} for total VFAs, and acetic, propionic and butyric acids, respectively. As for corn silage the results were: 80.0, 50.3, 19.2 and 12.7 mmol L^{-1}, respectively. Considering corn silage and sugarcane mixtures, at a 1:2 proportion, the results were 95.4, 61.7, 20.6 and 15.5 mmol L^{-1}, while at a 2:1 proportion (corn silage:sugarcane), the results for the VFAs mentioned above were 82.8, 50.7, 17.5, 11.1 mmol L^{-1}. The results in the present experiment (Table 1) differ from the ones obtained by Valvasori et al. (1998). The explanation for this may be related to the effects of using different

Table 1 - Comparison of average figures for volatile fatty acids (VFAs) and ammonia-nitrogen ($N\text{-}NH_3$) amounts, pH and dry matter degradability (DMD), for exclusive and combined roughages through the liquid fraction after *in vitro*/gas digestion.

	Volatile Fatty Acid										
Roughage	C_2	C_3	IC_4	C_4	IC_5	C_5	T-VFA	A:P	N-NH3	pH	DMD
	---------- mmol L^{-1} ----------								mg dL^{-1}		%
SC	35.0^{a}	12.1^{ab}	0.81^{a}	6.7^{abc}	1.3^{b}	1.1^{a}	56.9^{ab}	2.9:1	50.1^{cd}	5.7^{c}	60.6^{b}
SIL	33.1^{a}	13.3^{a}	1.02^{a}	6.7^{ab}	1.8^{a}	1.2^{a}	57.2^{ab}	2.5:1	54.0^{a}	5.9^{abc}	66.3^{a}
EG60	36.8^{a}	12.1^{ab}	0.91^{a}	5.8^{bcd}	1.5^{ab}	1.1^{a}	58.1^{ab}	3.0:1	49.4^{de}	5.9^{abc}	61.5^{ab}
EG180	26.7^{b}	8.7^{c}	0.91^{a}	5.0^{d}	1.5^{ab}	1.0^{a}	44.0^{c}	3.1:1	46.4^{f}	6.0^{ab}	34.6^{d}
SCSIL	37.0^{a}	13.3^{a}	0.91^{a}	7.5^{a}	1.6^{ab}	1.1^{a}	61.4^{a}	2.8:1	50.7^{bcd}	5.9^{abc}	63.9^{ab}
SCEG60	33.7^{a}	11.6^{b}	0.85^{a}	6.1^{bcd}	1.4^{ab}	1.1^{a}	54.7^{b}	2.9:1	47.5^{ef}	5.8^{bc}	60.4^{b}
SCEG180	27.3^{b}	9.7^{c}	0.73^{a}	5.5^{cd}	1.3^{b}	1.0^{a}	45.4^{c}	2.8:1	49.4^{de}	6.0^{abc}	48.6^{c}
SILEG60	34.0^{a}	12.8^{ab}	0.93^{a}	6.4^{abc}	1.5^{ab}	1.1^{a}	57.1^{ab}	2.7:1	53.1^{ab}	6.1^{a}	62.1^{ab}
SILEG180	34.2^{a}	12.2^{ab}	0.99^{a}	6.1^{bcd}	1.6^{ab}	1.2^{a}	55.9^{b}	2.8:1	52.3^{abc}	6.1^{a}	52.7^{c}
C.V. (%)	3.5	3.4	9.8	5.0	6.5	7.7	2.4	-	1.7	1.6	3.1
Value of F	20.41	31.56	2.14	11.04	4.88	2.46	39.89	-	24.44	6.59	212.14
Prob. > F	0.00023	0.00008	0.139	0.0012	0.015	0.1008	0.00005	-	0.00001	0.00067	0.00001

a, b, c, d, e, f – Averages followed by different letters within lines differ among them ($P<0.05$) by Tukey test.

C_2 – acetic acid, C_3 – propionic acid, IC_4 – isobutyric acid, C_4 – butyric acid, IC_5 – isovaleric acid, C_5 –valeric acid, A:P- acetic acid/ propionic acid ratio, T-VFA – total volatile fatty acids. (SC – sugarcane; SIL – corn silage; EG60 – 60-day elephantgrass, CV Taiwan 148; EG180- 180-day elephantgrass, CV Taiwan 148. Combined roughages at 50% DM proportion: SCSIL – sugarcane + corn silage; SCEG60 – sugarcane + 60-day elephantgrass; SCEG180 – sugarcane + 180-day elephantgrass; SILEG60- corn silage + 60-day elephantgrass; SILEG180 – corn silage + 180-day elephantgrass).

*The determination coefficient for the $N\text{-}NH_3$ standard curve was 0.999; C.V. – variation coefficient

concentrates in animal feeding, since the results for the analyzed roughages in the *in vitro*/gas system did not show this type of interference, representing only the relative concentration of the VFAs produced. As to the remaining VFAs of less nutritional importance for ruminants (IC_4 - isobutyric, IC_5 – isovaleric and C_5 – valeric), there were no differences ($P > 0.05$) among treatments (Table 1).

Lower concentrations of total volatile fatty acids (T-VFA), as well as of acetic and propionic acids, were found for the EG180 and SCEG180 roughages, whose values differed from the other roughages (Table 1) as function of the DM degradability profile, not only for the digestion residue but also for the total gas production (Table 1; Figure 1B). The fermentative pattern of such roughages produced lower energetic inputs, suggesting worse performances. According to Owens & Goetsch (1988), in a roughage diet, approximately 50 to 85% of the metabolizable energy used by ruminants comes from ruminal fermentation. The same authors related that VFA concentrations varied with total amounts generally falling between 60 and 150 mmol L^{-1}.

As to the relationship between acetic acid and propionic acid (A:P) productions for the evaluated treatments, no differences were found (Table 1). Such productions would probably be greater if concentrates had been included in treatments. These results are in accordance with those from Owens & Goetsch (1988), who stated that animals in starch-rich diets could have VFA concentrations averaging 200 mmol L^{-1}. Therefore, VFA in the ruminal liquid reflects the microbial activity and the absorption rate through the ruminal wall. The proportion of VFA varied as a function of the substrate type, intake level, feeding frequency, dilution rate and osmolarity (Owens & Goetsch, 1988).

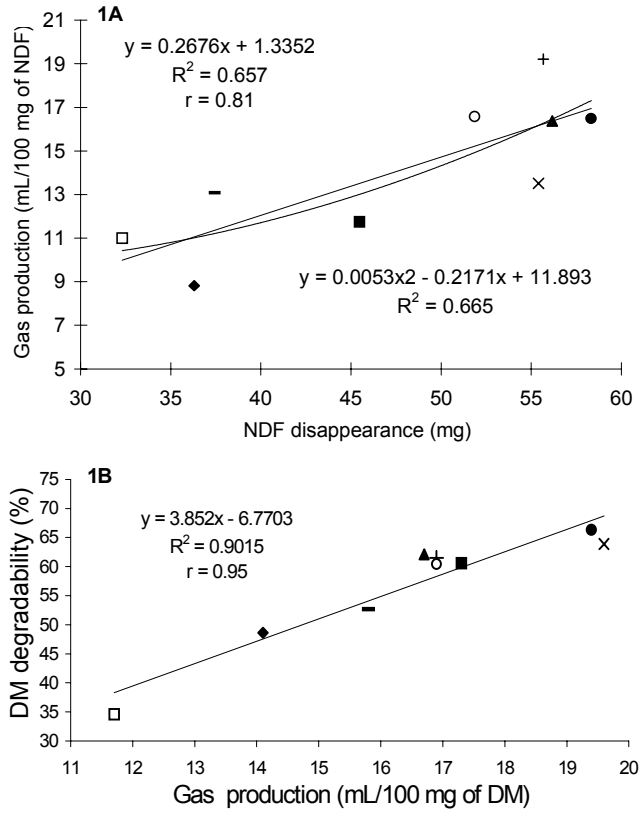

Figure 1 - Estimative of regression and correlations between NDF of disappearance and NDF gas production (1A), as well as between average gas production and dry matter degradability (1B), for all exclusive and combined roughages. (□ EG180; ♦ SCEG180; - SILEG180; ▲ SILEG60; O SCEG60; + EG60; ■ SC; ● SIL; x SCSIL).

In the present trial, the acetate/propionate (A:P) ratio from the DM digestion of corn silage, sugarcane and of sugarcane/corn silage mixture (50:50) were 2.5, 2.9 and 2.8, respectively. These values are similar to those found by Pires et al. (1999), who measured the 2.96, 3.27 and 2.91, for the three feeds respectively. The evaluation of volatile fatty acid concentration, after a 48-hour digestion period, can be used as a relative measurement in feed evaluation, taking into account that there is no VFAs absorption in the *in vitro*/gas system, unlike the rumen.

Results of $N\text{-}NH_3$ concentrations obtained for the *in vivo* experiment carried out by Pires et al. (1999) were about 40% of the ones in the present trial (25.5, 18.6 and 22.3 mg dL^{-1} for the *in vivo* experiment vs. 54.0, 50.1 and 50.7 mg dL^{-1} for the *in vitro*/gas experiment). The probable reason for the greater $N\text{-}NH_3$ concentrations in the *in vivo* experiment is that the *in vitro*/gas system, despite simulating the rumen environment, does not have the continuous nitrogen (N) recycling mechanism, salivation and buffering effect, which happen *in vivo.* Therefore, the *in vitro*/gas process, as a closed system, causes a metabolite concentration effect after 48-hour digestion, altering pH (Table 1) and causing microbial death.

The ammonia-nitrogen from true protein digestion and from non-protein nitrogen from feeds was an important nitrogen source for microbial growth. However, when there is little fermentable substrate, there is a decrease in microbial proliferation, which results in lower DM digestibility (Dryhurst & Wood, 1998). Such results, in the present experiment, were reflected by the DM degradability profile of the 180-day elephantgrass (EG180), which showed lower concentration of $N\text{-}NH_3$ (46.4 mg dL^{-1}) compared to corn silage (54.0 mg dL^{-1}). The fermentative pattern for these roughages affect negatively animal performance. For the microbial protein synthesis to occur effectively it is necessary that a proper energy source is available in the environment, since this source influences microorganism growth as well as the amount of $N\text{-}NH_3$ to be converted into microbial protein (Shirley, 1986).

All non-protein nitrogen can be used, depending on the total non-structural carbohydrates available for microbial growth and on the total ammonia nitrogen derived from dietary and salivary sources (Satter & Slyter, 1974). Thus, it is important to know the ammoniacal nitrogen concentration necessary to maximize the

microbial growth. The optimum N-NH_3 concentration in the ruminal liquid results from the maximum fermentation rate in the rumen or maximum microbial protein production generated by unit of fermented substrate (Mehrez et al., 1977). However, this concentration varies as a function of the energetic substrate type and availability, as well as with the pH during fermentation. In the present experiment, pH affected dry matter degradability depending on the readily fermentable substrate availability. This was evidenced in sugarcane fermentation, which showed worse results than the other treatments, probably due to high non-structural carbohydrate levels. Nonetheless, when sugarcane was combined with corn silage or grasses, no pH differences were found, probably due to the interactive effect of their physical and chemical compounds. This shows that pH was not a limiting factor for roughage DM digestion (Table 1).

Correlations between the disappearance of NDF (neutral-detergent fiber) and the NDF gas production in exclusive and combined roughages (Table 2; Figure 1A) were estimated, as well as the correlation between the average gas production and the average results for dry matter degradability for all analyzed roughages (Figure 1B). The greater the disappearance of NDF, the higher the NDF gas production (Figure 1A). Volatile fatty acid concentrations would probably tend to follow the same trend, as they are microbial fermentation by-products. On the other hand, VFA concentrations (Table 1) are related to dry matter degradability, which is directly affected by cell wall and cell content. Acetic, propionic acid and butyric acid concentrations, as well as T-VFAs concentration, varied linearly along with the dry matter degradability (Figure 2), similarly to results of NDF (Figure 1A). Although several roughages were being analyzed simultaneously, the VFA concentration was directly proportional to the dry matter degradability.

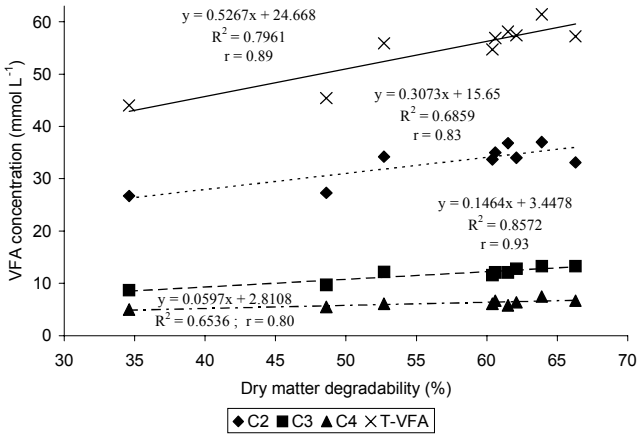

Figure 2 - Regression and correlations between dry matter degradability and volatile fatty acids concentrations for all exclusive or combined roughages. (C_2 – acetic acid, C_3 – propionic acid, C_4 – butyric acid and T-VFA – total volatile fatty acids).

Low correlation between gas production and the disappearance of NDF was recorded for sugarcane, corn silage and their mixtures, such as SCSIL and SILEG60, when analyzed individually (Table 2). A possible explanation for the low correlation for the treatments SC, SIL, SCSIL and SILEG60 (Table 2) may lie on the cell content proportion of the sample initial composition and on the sample composition after NDF isolation. These roughages had lower NDF concentration and higher cell content in their original composition (Table 2). However, during NDF isolation, phenolic substances responsible for the structural rigidity of fibers may have been released. Lower concentrations of such fibers may have prevailed in these roughages, causing distinct responses (non-linear) between gas production and the NDF disappearance. This was more evident in treatments involving corn silage (SIL, SCSIL, SILEG60), except for the SILEG180 mixture, which was influenced by the NDF, ADF (acid-detergent fiber) and lignin of the 180-day elephantgrass (78.3, 50.7 and 9.0%, respectively).

When correlations among all roughages were studied together, results were linear (R^2 = 0.66 and r = 0.81) (Figure 1A), in accordance with Pell & Schofield (1993), who mentioned the existence of high linear correlation between the disappearance of NDF and gas production. Roughages with high determination or correlation coefficients were those with high NDF levels (Table 2).

Table 2 - Estimative of regression and correlation between gas production and fiber disappearance in neutral-detergent fiber (NDF) of exclusive and combined roughages, through the *in vitro*/gas digestibility method during a 48-hour period.

Roughage	Regression of Equation	R^2	r
SC	Y = 0.2761 X - 0.8068	0.40	0.63
SIL	Y = 0.716 X + 12.307	0.07	0.30
EG60	Y = 2.7598 X - 134.42	0.97	0.98
EG180	Y = 1.3436 X - 32.437	0.99	0.99
SCSIL	Y = -0.3414 X - 32.432	0.21	0.46
SCEG60	Y = -3.2508 X - 185.18	0.86	0.93
SCEG180	Y = -0.2762 X - 18.846	0.95	0.97
SILEG60	Y = 0.3628 X - 4.0003	0.27	0.52
SILEG180	Y = 0.6791 X - 12.334	0.88	0.94

Exclusive roughages, NDF and NSC amounts, respectively: SC – sugarcane, 47.9% and 46.9%; SIL – corn silage, 51.3% and 32.3%; EG60 – 60-day elephantgrass, 69.5% and 17.4%; EG180 – 180-day elephantgrass, 78.3% and 15.3%.
Combined roughages at 50% DM proportion, NDF and NSC amounts, respectively: SCSIL – sugarcane + corn silage, 47.9% and 41.3%; SCEG60 – sugarcane + 60-day elephantgrass, 60.2% and 30.0%; SCEG180 – sugarcane + 180-day elephantgrass, 63.9% and 29.3%; SILEG60 – corn silage + 60-day elephantgrass, 60.1% and 25.1%; SILEG180- corn silage + 180-day elephantgrass, 62.1% and 26.6%. R^2 – determination coefficient, r = correlation coefficient, NSC = non-structural carbohydrates.

Roughage combination, at 50% DM proportion, therefore, may lead to positive or negative associative effects, depending on the fiber quality, non-structural carbohydrates proportion, starch and protein levels. The sugarcane/corn silage (50:50) mixture was an example of a positive associative effect, specially due its soluble fraction and starch, together with qualitative characteristics of the fiber and protein, which contributed to improve fermentation, probably leading to better animal performance.

No positive associative effect was recorded for the SCEG60 (50:50) mixture. However, its quality was maintained when compared to the original individual roughages. The negative associative effect concerning the VFAs was evident when sugarcane was combined with EG180, producing a lower-quality mixture ($P<0.05$), when compared to sugarcane (Table 1; Figure 1A), showing that fiber quality is a factor to be considered when combining roughages for posterior adjusts of the diet.

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