

Digestibility and microbial efficiency in steers fed diets based on corn silage hybrids and concentrate levels

[Digestibilidade e eficiência microbiana em novilhos alimentados com dietas à base de silagem de híbridos de milho e níveis de concentrado]

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ABSTRACT

A trial involving a 2x2 factorial design was conducted to evaluate the effect of corn silage hybrids and concentrate levels (25 and 50%) on intake and digestibility of nutrients, ruminal characteristics and microbial efficiency in steers. Four ruminal and abomasal cannulated steers (512±25kg of birth weight), were used in a 4x4 Latin square design. Treatments consisted of 75% silage A + 25% concentrate; 50% silage A + 50% concentrate; 75% silage B + 25% concentrate; and 50% silage B + 50% concentrate on dry matter (DM) basis. There were no differences in the intakes of DM, organic matter (OM), crude protein, and ether extract. The intake of non fiber carbohydrates and total digestible nutrients were positively affected by concentrate levels. The digestibility of DM and OM were also positively affected by concentrate levels. There were no effects of treatments on ruminal pH values, ruminal ammonia-N, and microbial efficiency.

Keywords: microbial protein synthesis, roughage, total digestible nutrient

RESUMO

Avaliou-se, em um esquema fatorial 2x2, o efeito de silagem de milho de diferentes híbridos e da porcentagem de concentrado (25 ou 50%) sobre o consumo e a digestibilidade de nutrientes, os parâmetros ruminais e a síntese de proteína microbiana em bovinos. Utilizaram-se quatro novilhos cruzados (HxZ) – (512±25kg) –, fistulados no rúmen e no abomaso, os quais foram distribuídos em um quadrado latino 4x4. Os tratamentos consistiram em 75% de silagem A + 25% de concentrado; 50% de silagem A + 50% de concentrado; 75% de silagem B + 25% de concentrado; e 50% de silagem B + 50% de concentrado em % da matéria seca (MS). Não houve diferença entre os consumos de MS, matéria orgânica (MO), proteína bruta e extrato etéreo, e houve efeito positivo de concentrado sobre os carboidratos não fibrosos e nutrientes digestíveis totais. A digestibilidade da MS e da MO foi positivamente influenciada pela porcentagem de concentrado. Não houve efeito de tratamento sobre o pH e a concentração de amônia ruminais, bem como sobre a eficiência de síntese microbiana.

Palavras-chave: nutrientes digestíveis totais, síntese de proteína microbiana, volumoso

INTRODUCTION

The feeding is the most expensive component of feedlot systems, mainly the concentrate ration cost. Thus, interactions and impacts of the use of different forage and concentrate ratios are extremely important to analyze the optimal relationship between cost and animal performance. In addition, the quality of silage used is also fundamental to get good economic results, because when using good quality silage, the utilization of concentrate can be decreased

and consequently the cost of the diet will be lower.

Whole-plant corn silage is a popular forage source for ruminants due to its high yielding properties, energy content, relatively high palatability, and easy incorporation into total mixed ration. Furthermore, the corn plants have high water soluble carbohydrate content, adequate lactic acid production and, consequently, good results in quality silage.

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However, hybrid, maturity, and moisture content are some of the factors that can alter the nutritive value of corn silage (Johnson *et al.*, 2002). Several studies have shown differences between hybrids in nutrient composition of whole plant corn and yield of dry matter (DM; Hunt *et al.*, 1993; Xu *et al.*, 1995; Melo *et al.*, 1999; Oliveira *et al.*, 2003; Qiu *et al.*, 2003). Commercial corn hybrids indicated for silage have been selected on the basis of agronomic traits such as grain yield and disease resistance (Clark *et al.*, 2002) and differences in the nutritive value of whole plant corn silages related to corn genetics have been ignored. Therefore, the choice of hybrid for silage should include agronomic traits and evaluation of nutritive value, as well as the intake and performance of animals fed those silages.

The commercial corn hybrids AGN35-A42 (Agromen) and A3663 (Bayer) are indicated for both corn grain and whole-plant corn silage although feeding trials to evaluate ruminal characteristics and microbial efficiency involving these corn silage hybrids at the same environment condition are limited. The objective of this study was to determine the effects of two corn silage hybrids and two concentrate levels on intake, digestibility, ruminal characteristics, and microbial efficiency in crossbred steers.

MATERIAL AND METHODS

Two corn hybrids, (Agromen, AGN35-A42, and Bayer, A3663) were used in this trial. Agromen (AGN35-A42) is a double cross hybrid and has an ultra-short-season life cycle while Bayer (A3663) is three-way cross hybrid with a short-season life cycle. Both corn hybrids are indicated for the production of grains and whole plant silage.

Diets were formulated to be isonitrogenous (13% crude protein, DM basis). Treatments were factorialized in a 2x2 arrangement and included the main effects of corn silage hybrids (Agromen, AGN35-A42 and Bayer, A3663) and concentrate levels (25 and 50%). Treatments consisted of 75% corn silage A + 25% concentrate (A25), 50% corn silage A + 50% concentrate (A50), 75% corn silage B + 25% concentrate (B25), 50% corn silage B + 50% concentrate (B50), on DM basis (Table 2).

Four Holstein x Zebu crossbred steers, averaging 512kg±25kg of birth weight (BW), and fitted with abomasal and ruminal cannulas, were used in a 4x4 Latin Square design to evaluate intake and apparent total tract and partial digestibility of nutrients, ruminal pH and ammonia-N, and microbial efficiency. Each experimental period had 18 d: 10 d for diet adaptation, 6 d to collect fecal and abomasal samples, 1 d for ruminal pH measurements and collection of ruminal fluid samples, and 1 d to collect ruminal contents to isolate bacteria. The experiment was conducted for 72 d (4 periods of 18 d). Steers were surgically fitted with ruminal and abomasal cannulae in agreement with techniques described by Leão and Coelho da Silva (1980).

Steers were randomly assigned to four dietary treatment sequences and fed individually *ad libitum* twice daily (07:00 and 15:00h). Diets were fed as total mixed ration, being corn silage and concentrate (previously mixed) weighed and mixed at feeding time. Orts were collected and weighed once daily and the feed offered was adjusted daily to yield Orts of about 5 to 10% of total offered. Animals had free access to water at all times. Feed ingredients and Orts were sampled daily and composed by weight for each steer within each period.

Feces and abomasal digesta samples (approx. 200g and 500mL, respectively) were collected between the d 11 and 16 of each period with intervals of 26h between the samplings. Indigestible acid detergent fiber (iADF) was used as an internal marker to estimate apparent nutrient digestibility and fecal and abomasal output. After drying at 60°C for 72 h, feed, Orts, and fecal and abomasal samples were ground to pass a 1-mm screen (Willey mill) and period composites per steer were prepared.

Ruminal contents (100mL) were obtained at 0, 1, 2, 4, 6 and 8 h after the morning feeding on d 17 of each period and subsequently strained through 2 layers of cheesecloth. pH was measured immediately. The ruminal fluid was preserved through the addition of 1mL of 9 M H₂SO₄, and stored at -20°C for analyses of NH₃-N concentration.

On d 18, the rumen contents were obtained 4h post-feeding and squeezed through two layers of cheesecloth to yield about 1,500mL of strained

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fluid. Particles retained on the cheesecloth were mixed with 500mL of 9g of NaCl/L, blended for 1min, refiltered through cheesecloth, and added to the 1.5L ruminal fluid sample. Bacteria were isolated by differential centrifugation (500g and 27,000g) according to procedures by Cecava *et al.* (1990). The resulting bacterial pellets were dried at 60°C for 48h and ground in a ball mill. The dried bacterial samples were ground and analyzed for DM, ash, and total N, and total purines were determined as proposed by Ushida *et al.* (1985).

The composite sample for each material (silage, concentrate, orts, abomasal digesta, and feces) was used to determine the DM (method #934.01 (Official..., 1990); organic matter determined by ash (method #924.05 (Official..., 1990); crude protein (CP) obtained by total N determination using the micro Kjeldahl technique (method #920.87 (Official..., 1990) and a fixed conversion factor (6.25); ether extract (EE) determined gravimetrically after extraction using petroleum ether in a Soxhlet instrument (method #920.85 (Official..., 1990); neutral detergent fiber (NDF) Van Soest *et al.*, 1991); ADF (method #973.18 (Official..., 1990), and sulfuric acid lignin (Van Soest *et al.*, 1991). The iADF (ADF remaining after a 144 h in situ incubation in a rumen-cannulated cow) was determined according to Cochran *et al.* (1986).

Non-fiber carbohydrates (NFC) were calculated by difference as shown in Equation 1

$$\text{NFC} = 100 - [\% \text{CP} + \% \text{NDF} + \% \text{EE} + \% \text{ash}] \quad [1]$$

Apparent total digestible nutrients (ApTDN) were calculated as shown in Equation 2 (NRC, 2001).

$$\text{App TDN} = (\text{digestible CP} + \text{digestible NDF} + \text{digestible NFC} + (2.25 \times \text{digestible EE})) / \text{DMI} \quad [2]$$

The determinations of the contents of NH₃-N of ruminal and abomasal fluid samples were done according to the original procedures by Fenner (1965) and adaptations by Vieira (1980). To quantify microbial protein and subsequently determine microbial efficiency, approximately 400mg of dry abomasal digesta samples were used, which were also analyzed for purines according to Ushida *et al.* (1985).

Data of intake, digestibility, and microbial efficiency were analyzed with the GLM procedure of SAS assuming a 4x4 Latin square design with a 2x2 factorial arrangement of treatments. The ruminal characteristics data collected over time were analyzed as repeated measures (Kuehl, 2000) using the MIXED procedure by SAS (Statistical..., 1990). When treatment interacted ($P < 0.05$) with sampling time, variables were analyzed within time periods. The variance-covariance structure AR(1) was used for estimating covariances. Differences were considered to be significant when $P \leq 0.05$.

RESULTS

The chemical composition of silages is presented in Table 1. Both corn silage hybrids had similar nutrient values, except for DM and NFC, which were numerically lower in corn silage hybrid A than those observed in corn silage hybrid B. In addition, corn silage A had a pH value of 3.6 and N-NH₃/Total N of 8.4% while corn silage B had a pH of 3.5 and N-NH₃/Total N of 6.3%.

Table 1. Chemical composition of corn silages

Item	Corn silage A	Corn silage B
DM, %	28.01	32.45
OM, % of DM	96.12	96.02
CP, % of DM	7.00	6.02
NDF, % of DM	53.00	51.14
iADF, % of DM	12.50	11.80
EE, % of DM	2.25	2.02
NFC, % of DM	33.87	36.86
Lignin, % of DM	3.62	3.45
pH	3.60	3.50
NH ₃ -N, % total N	8.40	6.30

DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; iADF = indigestible ADF; EE = ether extract and NFC = non-fiber carbohydrates.

The nutrient composition of the diets is shown in Table 2. As expected, diets with 50% concentrate provided higher amounts of DM, NFC and TDN than diets with 25% concentrate. The intake of nutrients is shown in Table 3. There were no

treatment effects ($P>0.05$) on the intakes of DM, OM, CP, and EE. The intakes of NDF, NFC and TDN were influenced ($P<0.05$) by concentrate levels.

Table 2. Ration composition and analyzed content in diets

Item	Corn Silage A		Corn Silage B	
	25	50	25	50
Corn silage	75.0	50.0	75.0	50.0
Ground corn	14.86	41.0	14.86	41.0
Cottonseed meal	8.27	7.03	8.27	7.03
Urea	1.00	1.00	1.00	1.00
Ammonium sulfate	0.10	0.10	0.10	0.10
Sodium chloride	0.25	0.25	0.25	0.25
Calcite limestone	0.50	0.60	0.50	0.60
Mineral premix ¹	0.02	0.02	0.02	0.02
Nutrient content in diets				
DM, %	43.53	59.38	46.91	61.60
OM, % of DM	94.28	94.44	94.20	94.39
CP, % of DM	13.06	13.13	12.32	12.64
NDF, % of DM	43.74	32.73	42.35	31.80
iADF, % of DM	10.58	7.45	10.06	7.10
EE, % of DM	2.14	2.15	1.95	2.02
NFC, % of DM	38.81	50.46	41.04	51.95
Lignin, % of DM	3.16	2.46	3.03	2.37

¹Composition (%): cooper sulfate (22.50), cobalt sulfate (1.40), zinc sulfate (75.40), potassium iodate (0.50), sodium selenite (0.20). DM= dry matter; OM = organic matter; CP = crude protein; NDF= neutral detergent fiber; ADF = acid detergent fiber; iADF = indigestible ADF; EE = ether extract; and NFC = non-fiber carbohydrates.

Table 3. Nutrient intake according to corn silage hybrids and concentrate levels

Item	Corn silage A		Corn silage B		SEM	<i>P</i> -value		
	25	50	25	50		Silage	Conc	S x C
Intake, kg/d								
DM	10.97	11.12	10.22	11.79	0.62	0.94	0.21	0.30
OM	10.49	10.71	9.78	11.44	0.59	0.99	0.16	0.27
CP	1.48	1.47	1.32	1.53	0.08	0.55	0.24	0.17
EE	0.24	0.22	0.21	0.23	0.02	0.64	0.83	0.33
NDF	4.61	3.23	4.17	3.29	0.35	0.61	0.02	0.49
NFC	4.46	6.07	4.17	6.44	0.23	0.87	<0.001	0.21
TDN	7.38	8.13	6.62	8.45	0.47	0.66	0.03	0.29
Intake, % of BW								
DM	1.95	1.98	1.80	2.08	0.11	0.81	0.20	0.30
NDF	0.82	0.58	0.74	0.58	0.06	0.54	0.02	0.51

DM= dry matter; OM = organic matter; CP= crude protein; EE= ether extract; ndf= neutral detergent fiber; NFC = non-fiber carbohydrates; TDN= total digestible nutrients.

S = silage source effect; C= concentrate level effect; S x C = silage and concentrate interaction effect.

The digestibility of nutrients is shown in Table 4. Total tract apparent digestibility of DM and OM, as well as a % of TDN were affected by concentrate levels ($P<0.05$). The ruminal tract digestibility of nutrients was similar ($P > 0.05$) among all treatments (Table 5). There were no

effects of corn silage hybrids on the digestibility of all nutrients in both experiments. There were no treatment effects on ruminal pH values and ruminal ammonia-N concentrations ($P>0.05$), and the means are shown in Table 6.

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Table 4. Apparent total tract digestibility of nutrients and % of total digestible nutrients according silage hybrids and concentrate levels

Item ^a	Corn silage A		Corn silage B		SEM	Silage	P-value ^b	
	25	50	25	50			Concentrate	S x C
DM	66.22	71.41	64.05	71.55	2.60	0.71	0.05	0.67
OM	66.80	72.11	64.68	72.44	2.57	0.74	0.04	0.65
CP	66.77	66.88	65.87	66.81	2.51	0.85	0.84	0.87
EE	78.94	76.85	78.89	79.47	2.42	0.61	0.77	0.60
NDF	53.96	49.35	53.52	54.10	3.21	0.53	0.55	0.45
NFC	79.36	85.30	75.77	81.61	2.52	0.20	0.06	0.98
TDN	67.91	73.22	64.97	71.64	2.44	0.39	0.05	0.79

^aValues are expressed as %; DM= dry matter; OM= organic matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fiber; NFC= non-fiber carbohydrates; TDN= total digestible nutrients; S = silage source effect; C= concentrate level effect; S×C = Silage and concentrate interaction effect.

Table 5. Apparent ruminal tract digestibility of nutrients according to silage hybrids and concentrate levels

Item ^a	Corn silage A		Corn silage B		SEM	Silage	P-value	
	25	50	25	50			Concentrate	S x C
	Ruminal tract digestibility							
DM	83.2	82.1	84.1	82.4	1.88	0.74	0.48	0.88
OM	84.3	82.7	84.9	83.2	1.73	0.73	0.39	0.98
CP	55.60	58.06	53.35	60.92	3.36	0.93	0.17	0.48
EE	-18.95	-28.38	-13.47	-21.67	19.82	0.77	0.67	0.98
NDF	91.3	95.4	96.9	95.8	1.75	0.13	0.41	0.19
NFC	89.9	87.6	86.8	86.9	2.53	0.50	0.67	0.67

^aCalculated as % of digested. DM= dry matter; OM= organic matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fiber; NFC= non-fiber carbohydrates; S = silage source effect; C= concentrate level effect; S×C = silage and concentrate interaction effect.

Table 6. Means values of ruminal pH and ammonia-N concentration after feeding time (hours)

Time (hours)	Corn silage A		Corn silage B		Means
	25	50	25	50	
	pH				
0	6.38	6.18	6.20	6.04	6.20
2	6.29	6.09	6.02	5.85	6.05
4	5.88	5.90	6.02	6.01	5.95
6	5.92	5.92	5.90	5.78	5.88
8	6.23	5.97	6.01	5.95	6.03
	NH ₃ -N (mg/100mL)				
0	9.18	11.55	9.18	10.50	10.10
2	24.49	20.12	22.48	25.80	23.22
4	21.52	22.74	17.64	19.46	20.34
6	18.67	17.76	14.43	12.60	15.86
8	13.12	11.35	10.13	10.74	11.34

Table 7 shows the data of microbial N efficiency. There was a concentrate effect on rumen-degraded organic matter (RDOM) ($P=0.02$) and rumen-degraded carbohydrate (RDCHO) ($P=0.04$). However, the efficiency of

microbial synthesis, g of microbial N/kg RDOM, g of microbial N/kg RDCHO and g of microbial crude protein/kg TDN were not affected ($P>0.05$) by treatments.

Table 7. Microbial N efficiency according to corn silage hybrids and concentrate levels

Item	Corn silage A		Corn silage B		SEM	P-value		
	25	50	25	50		Silage	Conc	S x C
Rumen degradability, kg/d								
OM	5.85	6.39	5.36	6.90	0.33	0.98	0.02	0.18
CHO ^a	5.39	5.93	4.96	6.26	0.36	0.90	0.04	0.33
Microbial N efficiency								
g mic N/kg RDOM ^b	22.29	20.09	23.71	18.80	1.86	0.97	0.11	0.50
g mic N/kg of RDCHO ^c	24.28	21.70	25.59	20.74	2.11	0.94	0.13	0.61
g mic CP/kg of TDN	110.3	98.2	120.2	95.1	10.04	0.75	0.11	0.54

OM= organic matter; CHO= carbohydrates; CP= crude protein; EE= ether extract; TDN= total digestible nutrients.

^aCHO = 100 - (%CP + %EE + %Ash), Sniffen *et al.* (1992); RDOM = ruminally degraded organic matter; RDOM (kg/d) = Intake of OM - abomasal OM flow; ^cRDCHO = ruminally degraded carbohydrates: RDCHO (kg/d) = Intake of CHO - abomasal CHO flow; S = silage source effect; C= concentrate level effect; S×C = silage and concentrate interaction effect.

DISCUSSION

Overall, both silages had similar nutrient contents, except for a % of DM, NDF and NFC (Table 1). Freitas *et al.* (2003) also observed no effect of silages of five corn genotypes on DM intake and digestibility. According to Muck and Pitt (1993), both silages had good quality due to their adequate percentage of N-NH₃/Total N and pH values. The diets with 50% concentrate in association with either corn silage hybrids provided higher amounts of DM, NFC and TDN and lower NDF than 25% concentrate diets, certainly due to a higher percentage of corn ground in those diets.

Although some authors have found linear (Dias *et al.*, 2000; Souza *et al.* 2002; Pereira *et al.*, 2006) or quadratic (Verás *et al.*, 2000; Ítavo *et al.*, 2002; Silva *et al.*, 2005; Costa *et al.*, 2005) increases in DM intake with an increase of concentrate levels in beef cattle diets, no effects of treatments were observed in this study. Moraes *et al.* (2002) also found no increase in DM intake with the concentrate levels. These variations among studies probably occurred due to other variables related to the kind of animal, age of animal, climatic conditions, days of feeding, etc, which can affect DM intake.

The higher intake of NFC and TDN and lower intake of NDF in steers fed diets with 50% concentrate likely occurred as a result of higher amounts of ground corn in these diets than those with 25%. Similar results were found by Costa *et al.* (2005) who evaluated the effects of concentrate levels (5, 35, and 65%, DM) on

nutrient intake in Nelore steers. The increases observed in DM and OM digestibility in diets with 50% concentrate likely occurred due to a higher amount of non-fiber carbohydrates in those diets than in 25% concentrate diets. In general, the apparent digestibility of NFC is higher than the digestibility of structural carbohydrates, so the replacement of corn silage with corn ground in 50% concentrate diets provided more NFC to those diets than in 25% concentrate diets. Similar results were observed by Cardoso *et al.* (2000), Ítavo *et al.* (2002), Putrino *et al.* (2007) and Chizzotti *et al.* (2010).

NDF apparent digestibility was not affected by concentrate levels, which can be explained by lack of differences in ruminal pH values (Tab.4). Thus, cellulolytic microbes were not affected by ruminal pH and, consequently, fiber digestion was not influenced. Pereira *et al.* (2006) and Costa *et al.* (2005) observed linear decreases in NDF digestibility as concentrate levels in beef cattle diets increased. The ruminal digestibility of all nutrients was similar among all treatments.

Ruminal pH values were not affected either by silage or by concentrate levels, which means that the ruminal environment was adequate for microbial growth. Overall, the mean ruminal pH value observed was 6.02, which is greater than the 5.0 to 5.5 range that was suggested by Hoover (1986) in which ruminal digestibility of fiber is negatively affected.

In the same way, NH₃-N concentration was also not affected by treatments and averaged 16.2mg/100mL. During all time sampling the

ruminal NH₃-N concentrations were above levels (5 mg/100mL) recommended by Satter and Slyter (1974) to optimize ruminal digestion. Pereira *et al.* (2006) also found no effect of different concentrate levels on ruminal pH and ruminal NH₃-N concentrations when steers were fed with sorghum silage and different concentrate levels.

Concentrate levels affected the rumen-degraded OM ($P=0.02$) and rumen-degraded carbohydrate ($P=0.04$) which were higher in steers fed 50% concentrate diets than in those fed 25%. This increase of ruminal degradability certainly occurred due to higher amounts of ground corn in those diets with more concentrate, which provided more readily fermentable carbohydrates.

Bacterial growth is largely dependent on the amount of ammonia and fermentable organic matter available in the rumen (Bryant and Robinson, 1962). Readily fermentable carbohydrates, such as starch or sugars, are more effective than other carbohydrate sources, such as cellulose, in promoting microbial growth (Stern and Hoover, 1979). Although rumen-degraded OM and rumen-degraded CHO were higher in 50% concentrate diets than in those with 25%, microbial N production (Table 8) was not affected by treatments. Moreover, microbial efficiency was also not influenced by treatments ($P>0.05$) when expressed on a rumen-degraded OM, rumen-degraded carbohydrate, or on a TDN basis. The mean microbial efficiency of 105.95g microbial CP/kg TDN is lower than the recommended by National... (1996), which is 130g microbial CP/kg TDN.

CONCLUSIONS

The association of both corn silage hybrids evaluated with 25 or 50% concentrate does not affect the ruminal pH and ruminal ammonia-N concentration, and microbial efficiency. Our findings suggest that, as the two corn silage hybrids evaluated had similar behavior on nutrient intake and microbial efficiency, despite concentrate level, the choice for the more adequate corn silage hybrid should include the agronomic traits.

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