Livestock Science 155 (2013) 71–76

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Intake and ruminal digestion determined using omasal and reticular digesta samples in cattle fed diets containing sugar cane *in natura* or ensiled sugar cane compared with maize silage

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ARTICLE INFO

Article history:
Received 19 December 2012
Received in revised form
12 March 2013
Accepted 2 April 2013

Keywords:
Calcium oxide
Intake
Digestibility

ABSTRACT

Sugar cane is widely used in an *in natura* forage in tropical countries, but the adoption of silage methods facilitates the preservation of its nutritional value and improves the logistics of its use. To explain differences in performance using alternative forages, it is important to conduct studies that evaluate the various digestion sites for the nutrients provided in diets. However, considering that the collection of omasal digesta is quite laborious and requires the use of a vacuum pump, reticular sampling has been suggested as a promising alternative. Thus, the objective of this study was to evaluate the intake and ruminal digestibility obtained from samples of digesta collected in the reticulum and omasum of cattle fed different diets. Five rumen-fistulated crossbred cattle with an average initial live weight of 336 ± 16.6 kg were used, being distributed in a 5×5 Latin square design. Five diets were evaluated, which contained 60% forage and 40% concentrate on dry matter basis using different forages: maize silage (CS); sugar cane *in natura* (SCIN); sugar cane silage (SCS0%); sugar cane silage treated with 0.4% calcium oxide (SCS0.4%) or 0.8% calcium oxide (SCS0.8%) on wet basis. The percentage of crude protein (CP) in all of the forages was corrected to 11% based on dry matter (DM) using a mixture of urea/ammonium sulfate (9:1). Six collections of reticular and omasal digesta were obtained over three days at 12 h intervals. To calculate the flow of reticular and omasal nutrients, a double marker system was employed, using cobalt–EDTA and indigestible neutral detergent fiber (NDFi) as markers. The reticular and omasal digesta were similar ($P > 0.05$) to estimate ruminal digestibility of DM, organic matter (OM), CP, neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC). However, the ruminal digestibility of ether extract (EE) and the intestinal digestibility of CP and EE differed ($P < 0.05$) between sampling sites. The results indicate that the omasal digesta is more suitable than the reticular digesta for measuring the ruminal digestion of diet components.

1. Introduction

In Brazil, the high portion of forage in diets for ruminants is attributed to the lower cost of this component in the diet. Maize silage has a high nutritional value and is widely used in feedlots because it increases animal performance. Sugar cane is also traditionally used in cattle diets because this crop is less expensive than maize silage and its production coincides with the period of increased scarcity of forage in the country. In addition, sugar cane has an inferior nutritional value when compared with maize silage.

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http://dx.doi.org/10.1016/j.livsci.2013.04.003

Sugar cane is widely used in an *in natura* or unprocessed form, but the adoption of silage methods facilitates the preservation of its nutritional value and improves the logistics of its use. Chemical additives, like calcium oxide, are utilized in sugar cane silage to reduce losses from processing and improve the fermentation standards and the digestibility of the sugar cane fiber, which together represent the major limitations of the use of this type of forage, impacting animal performance.

To explain differences in performance, it is important to conduct studies that evaluate the various digestion sites for the nutrients provided in diets and the rates of fiber digestion. Different sampling methods and marker systems are currently used to estimate ruminal flow (Krizsan et al., 2010). However, considering that the collection of omasal digesta is quite laborious and requires the use of a vacuum pump, reticular sampling has been suggested as a promising alternative to omasal sampling because, according Krizsan et al. (2010), it involves less interference with the animals themselves.

Therefore, the aims of this study were to evaluate the ruminal digestibility of dry matter and other constituents in the diet using samples obtained from the omasum and reticulum and to evaluate intake and digestibility of sugar cane *in natura* or ensiled sugar cane compared with maize silage for beef cattle.

2. Materials and methods

All procedures involving animals were approved by the Brazilian committee for animal care and experimentation.

2.1. Animals, experimental design, and diets

This study was conducted in the Department of Animal Science at the Universidade Federal de Viçosa, located in Viçosa, MG, Brazil. Five rumen-fistulated crossbred cattle (Holstein $\times$ Zebu) with an average initial live weight of 333 ± 17 kg were used in the experiment, distributed in a 5×5 Latin square design. The experiment lasted 105 days, corresponding to five 21-day periods. These periods were sub-divided into periods of nine days, during which the animals were allowed to adapt to the experimental diets, location and gas collector apparatus, and 12 days, during which collections were performed.

The animals were weighed, identified and vermifuged prior to the experiment and housed in individual pens (8 m²) fitted with feeders and waterers.

A total of five diets containing 60% forage and 40% concentrate based on dry matter (DM) and containing different forages were tested: maize silage (CS); sugar cane *in natura* (SCIN); a sugar cane silage control (SCS0%); sugar cane silage treated with 0.4% calcium oxide (SCS0.4%); and sugar cane silage treated with 0.8% calcium oxide (SCS0.8%), based on wet basis. The percentage of crude protein (CP) in all of the forages was corrected to 11% by the daily addition of a mixture of urea/ammonium sulfate (9:1) to the forage based on the DM content in the forage.

From Monday to Friday, the *in natura* sugar cane was cut and chopped daily and then provided to the animals. The *in natura* sugar cane provided on weekends was crushed on Friday morning and maintained in stacks over the weekend. This system was based on a report by Menezes et al. (2011) that indicated that animal performance is not altered when sugar cane is chopped and stored for three days before being fed to animals.

Isoproteic diets with approximately 12% crude protein were used. The proportion of the ingredients in the concentrate mixture and the chemical composition of the diets are shown in Table 1. In the diets SCS0.4% and SCS0.8%, limestone ($CaCO_3$) was substituted for sand in the same proportions, in order to not extrapolate calcium requirements.

2.2. Experimental procedures and sampling

Forage provided and leftovers were sampled daily during the collection period and subjected to partial drying in a forced ventilation oven set at 60 °C for 72 h. The ingredients that comprised the concentrate were sampled directly from the feed mill silos on the days that they were mixed.

Table 1
Percentages of ingredients used in the concentrate and in the experimental diets and the compositions of the concentrate and the diets.

Parameter	Concentrate	Diets
DM (g kg^{-1})		
Forage	–	600.0
Corn grain	851.0	340.0
Soybean meal	131.0	52.89
Calcium phosphate	7.6	3.40
Limestone or sand[a]	6.6	3.0
Salt	3.2	0.4
Premix[b]	0.6	0.03

Parameter	Conc.	Diets				
		CS[c]	SCIN[d]	SCS0%[d]	SCS0.4%[d]	SCS0.8%[d]
Chemical composition of concentrate and diets in DM basis						
DM (g kg^{-1})	872.4	533.2	532.0	524.3	525.1	533.3
In g kg^{-1} DM						
OM	962.2	962.5	969.0	953.9	945.5	937.6
CP	137.3	120.0	121.3	121.5	122.8	121.9
EE	30.1	26.3	23.0	19.6	19.1	22.4
NDF	94.8	322.8	302.4	391.5	358.9	367.2
NFC	700.0	509.0	552.0	451.7	476.0	456.5
NDFi	7.12	119.26	159.71	203.62	189.85	191.19
Lignin	12.4	33.52	38.82	62.86	55.26	65.65
pH[e]	–	3.53	–	3.39	3.84	4.14

Conc.—concentrate; CS—corn silage; SCIN—sugar cane *in natura*; SCS0%—untreated sugar cane silage; SCS0.4%—sugar cane silage treated with 0.4% calcium oxide; SCS0.8%—sugar cane silage treated with 0.8% calcium oxide; DM—dry matter; OM—organic matter; CP—crude protein; EE—ether extract; NDF—neutral detergent fiber corrected for ash and protein; NFC—non-fiber carbohydrates; NDFi—indigestible neutral detergent fiber.

[a] For the diets containing sugar cane silage treated with 0.4% and 0.8% calcium oxide limestone was substituted for washed and dried sand.

[b] Chemical composition of the premix: 2.1 g kg^{-1} of cobalt sulfate. 167.8 g kg^{-1} of copper sulfate. 3.59 g kg^{-1} potassium iodate. 262.3 g kg^{-1} of manganese sulfate. 0.93 g kg^{-1} sodium selenite. 563.3 g kg^{-1}of zinc sulfate.

[c] 16.5 g of urea+ammonium sulfate per kg DM of forage intake.

[d] 32.0 g urea+ammonium sulfate per kg DM of forage.

[e] For pH measurement, 25 g humid sample was processed with 225 ml of ringer solution, for 1 min. The pH was measured in the water extract.

Six collections of reticular and omasal digesta were performed at 12-h intervals between day 15 and day 18. On day 15, samples were collected at 6:00am and 6:00pm. On day 16, they were collected at 10:00am and 10:00 pm. Samples were collected at 2:00 pm on day 17 and at 2:00 am on day 18. At these same sample collection times, approximately 200 g of feces was collected from each animal, and the indigestible neutral detergent fiber (NDFi) content was used to estimate the production of fecal DM. Fecal samples were partially dried in a forced ventilation oven at 60 °C. Subsequently, these samples were milled to a size of 1–2 mm in a Wiley-type laboratory mill.

The collection of reticular digesta was performed using a 250 mL flask with a screw-cap lid that was introduced with the lid closed via the rumen until it reached the reticulum. The lid was then removed in the reticulum, and after filling the flask with reticular digesta, the lid was closed while still within the reticulum, and the flask was removed, forming part of the sample to be collected. This procedure was repeated four times until a total of 1 L of reticular digesta was collected.

To collect the omasal digesta, the technique reported by Huhtanen et al., (1997) was adapted as follows: The collection of omasal digesta was performed by introducing the end of a collection tube into the rumen and passing it into the reticulum–omasal orifice until the first part of the tube passed into the orifice, where it was secured by hand during the collection period. The other end of the collection tube was fitted to one of the openings of a kitassato flask, and a vacuum pump was attached to the other opening. The vacuum pump was subsequently turned on to begin collection, and the digesta was collected through the tube via suction until it reached the kitassato flask. Approximately 1 L of digesta was obtained per collection.

After collection, the reticular and omasal digesta samples were frozen (−20 °C) until processing. At the end of each experimental period, these samples were thawed at room temperature and filtered through a 100-μm nylon filter with pores covering 44% of the surface (Sefar Nitex 100/44, Sefar, Thal, Switzerland) to generate two phases: the filtrate, which corresponded to the liquid phase and small particles, and the residue, which corresponded to the large particle phase. Subsequently, these samples were dried in a forced ventilation oven at 60 °C for 72 h, milled in a Wiley-type laboratory mill, and stored for subsequent use. The flow of DM and the constituents of the reticular and omasal digesta were calculated as described by France and Siddons (1986). To calculate the flow of reticular and omasal nutrients, a double marker system was employed in which cobalt–EDTA (6 g/day divided in four doses were infused in rumen of each animal beginning three days before reticular and omasal digesta sampling) was used as the liquid phase and a small particle marker and NDFi were used as particulate phase.

On day 19, the rumen was completely emptied 4 h after the morning diet was provided to determine the rates of indigestion and ruminal pool for each diet using the technique described by Allen and Linton (2007). On day 21, the rumen was emptied immediately before feeding. After emptying the rumen, the total weight of the digesta was determined, followed by filtering through four layers of cheesecloth to separate the solid mass and liquid phases. A representative sample of both phases was collected to evaluate the DM, NDF and NDFi contents. After sampling, the phases were again mixed, and the remaining digesta was returned to the rumen. The rate of ingestion (k_i) was calculated by dividing the daily intake of DM or other components by their respective ruminal pool (Allen and Linton, 2007).

At the end of the sampling period, the fecal, reticular digesta and omasal digesta samples were partially dried at 60 °C for 72 h and milled to produce 1 and 2 mm slices in a laboratory mill. At the end of each period, a composite sample was prepared from the leftovers, feces and omasal and reticular digesta based on the air-dried weight of the samples for each animal, and the samples were then properly identified and stored in plastic containers for further analysis.

2.3. Chemical analysis

Samples of forage, leftovers, the ingredients in the concentrate, feces and reticular and omasal digesta were analyzed for DM by drying the samples at 105 °C for 12 h in a forced air oven. Samples were also analyzed for ash and nitrogen content according to the 942.04 and 976.05 methods, respectively, of AOAC (1990). Analyses of neutral detergent fiber (NDF), and lignin (sa) (Van Soest, 1991) were also done. Heat stable amylase and sodium sulfite were used in the NDF procedure, and the results of NDF were expressed on ash-free basis. Sulfuric acid method was used to analyze lignin (sa). The reticular and omasal digesta samples were analyzed to determine cobalt levels using an atomic absorption spectrophotometer. The NDFi content was calculated after incubating the fecal, reticular and omasal digesta, forage and concentrate samples, which were milled to 2 mm, in F57 bags (Ankom®) in situ for 288 h as described by Valente et al. (2011) for tropical forages.

The quantification of non-fiber carbohydrates (NFC) was performed according to Detmann and Valadares Filho (2010) as follows: NFC = 100−[(%CP−%CP of urea+%urea)+%NDFap+%EE+%MM], where NDFap = neutral detergent fiber corrected for ash and protein.

2.4. Statistical analysis

The relative data on intake and total digestibility were analyzed using the MIXED procedure in SAS (version 9.1). The means were compared with the Tukey test. A significance value of 0.05 was adopted as the critical value of the probability of type I error.

Ruminal digestibility was analyzed via repeated measures in space using the MIXED procedure in SAS (version 9.1) according to the following model:

$$Y_{ijkl} = \mu + D_i + a_j + p_k + e_{ijk} + L_l + DL_{il} + \varepsilon_{ijkl}$$

where μ is a general constant; D_i is the effect of diet i; a_j is the effect of animal l; p_k is the effect of the experimental period k; and e_{ij} is the residual random effects between plots; L_l is the effect of sampling location l; DL_{il} is the effect of the interaction between diet i and sampling location l; and ε_{ijkl} is the unobservable random error, assuming a normal distribution.

3. Results

Diets based on maize silage resulted in a greater ($P<0.05$) nutrient intake in kg day^{-1} and g kg^{-1}of body weight (BW) (Table 2). The intake recorded for diets containing sugar cane silage was similar ($P>0.05$). There was no difference in the intake of NDFi between diets ($P>0.05$).

Diets containing maize silage and sugar cane *in natura* exhibited an apparently similar digestibility of DM, OM, CP, EE, NDF and total digestible nutrients (TDN) contents ($P>0.05$) (Table 2). The digestibility of the constituents in sugar cane silage was similar.

There was no interaction ($P>0.05$) between sampling location and the treatments for any of the analyzed variables (Table 3). The ruminal digestibility for DM, OM, CP, NDF and NFC was similar ($P>0.05$) for the digesta collected in the omasum and the reticulum. There was an effect of the sampling site ($P<0.05$) detected for the ruminal digestibility of EE, which was lesser in the digesta collected in the omasum. When the effects of the different diets were evaluated, no difference was observed in the ruminal digestibility of DM, NDF and NFC. The ruminal digestibility of EE was lower ($P<0.05$) for the maize silage diet compared to the other diets. The intestinal digestibility of CP and EE was found to be greater ($P<0.05$) in the omasal digesta than in the reticular digesta.

There was no effect ($P>0.05$) of the treatment on the rate of ingestion and ruminal pool of NDFi (Table 2). Diets containing maize silage exhibited NDFpd ruminal pool greater than diets based on sugar cane silage treated with 0.4% and 0.8% calcium oxide.

4. Discussion

The greater intake of nutrients observed in the diets based on maize silage can be explained by the lesser NDFi content (Table 1) and the higher rate of ruminal digestion of the potentially digestible fiber fraction of this diet, resulting in higher rates of passage of the digesta through the gastrointestinal tract and a consequent increase in intake by the animals.

The addition of alkaline products such as calcium oxide to sugar cane silage is recommended to increase the digestibility of this type of feed and consequently its intake, by altering the chemical structure of the silage through a process known as alkali swelling of cellulose, which, according to Jackson (1977), consists of the

Table 2
Mean values for the intake, the total apparent digestibility coefficients and TDN content, rates of ingestion and pool sizes of the diet constituents in crossbred cattle fed diets based on sugar cane *in natura* and ensiled sugar cane or corn silage.

Parameter	Diets						
	CS	SCIN	SCS0%	SCS0.4%	SCS0.8%	SEM	*P*
Intake(kg day^{-1})							
DM	7.11 a	5.23 b	3.87 b	4.29b	4.08 b	0.26	0.0024
OM	6.82 a	5.05 b	3.72 b	4.15 b	3.80 b	0.24	0.0020
CP	0.902 a	0.696 b	0.516 c	0.580 bc	0.570 bc	0.05	0.0035
EE	0.188 a	0.144 b	0.086 c	0.107 bc	0.094 c	0.007	0.0015
NDF	2.19 a	1.42 b	1.41 b	1.41 b	1.17 b	0.08	0.0038
NFC	3.71 a	2.96 b	1.84 c	2.20 bc	2.12 c	0.14	0.0014
TDN	5.45 a	4.03 b	2.95 c	3.40 bc	2.85 c	0.17	<0.001
NDFi	0.898	0.818	0.728	0.726	0.560	0.04	0.0523
Intake (g kg^{-1}of body weight)							
DM	20.8 a	14.8 b	11.8 b	9.6 b	12.5 b	0.08	0.0051
NDFap	6.41 a	3.99 b	4.32 b	3.23 b	3.60 b	0.02	0.0071
NDFi	2.61	2.39	2.25	1.70	1.71	0.01	0.1572
Total apparent digestibility (%)							
DM	68.6	70.6	69.6	72.1	72.0	1.08	0.8176
OM	70.5	72.4	72.7	75.4	74.7	0.90	0.5091
CP	68.2 b	72.5 ab	76.8 a	78.7 a	80.3 a	1.02	0.0077
EE	79.3	81.1	75.8	83.2	83.8	1.64	0.3254
NDF	48.0	36.2	52.3	50.4	45.0	1.82	0.0599
NFC	86.2 b	90.7 a	88.4 ab	90.3 a	89.4 a	0.46	0.0316
TDN	69.8	73.4	73.7	77.0	74.5	1.02	0.3265
K_i (h^{-1})							
NDFi	0.0230	0.0184	0.0236	0.0238	0.0179	0.2675	0.0015
Ruminal pool (kg)							
NDFi	1.39	1.59	1.44	1.39	1.80	0.1577	0.0656
NDFpd	1.29 a	0.813 ab	0.820 ab	0.530 b	0.696 b	0.0138	0.076

CS—corn silage; SCIN—sugar cane *in natura*; SCS0%—untreated sugar cane silage; SCS0.4%—sugar cane silage treated with 0.4% calcium oxide; SCS0.8%—sugar cane silage treated with 0.8% calcium oxide; SEM—standard error of the mean; DM—dry matter; OM—organic matter; CP—crude protein; EE—ether extract; NDF—neutral detergent fiber corrected for ash and protein; NFC—non-fiber carbohydrates; NDFi—indigestible neutral detergent fiber; k_i—rate of dietary ingestion; NDFpd—neutral detergent fiber potentially digestible.
Means followed by different lowercase letters in the same row differ ($P<0.05$).

Table 3
Ruminal and intestinal digestibility expressed as a percentage of the quantity that reaches each site, as determined by collecting reticular and omasal digesta from cattle fed diets based on sugar cane and *in natura* or ensiled corn silage.

Parameter	CS		Average	SCIN		Average	SCS0%		Average	SCS0.4%		Average	SCS0.8%		Average		*P*		
	Ret	Om		Ret	Om		Ret	Om		Ret	Om		Ret	Om		SEM	Treat	Local	T × L
Ruminal digestibility																			
DM	39.40	33.20	36.30	36.20	41.60	38.90	36.00	43.20	39.60	36.67	39.67	38.17	41.00	51.00	46.00	0.01	0.2048	0.1130	0.2496
OM	52.20	43.20	47.70 B	49.00	52.40	50.70B	50.20	55.40	52.80B	52.29	53.79	53.04AB	58.40	63.80	61.10A	0.01	0.0124	0.6031	0.3282
CP	4.80	−13.20	−4.20 B	19.20	11.20	15.20AB	32.40	23.20	27.80A	17.45	15.20	16.32 AB	25.00	32.80	28.90A	0.02	0.0029	0.2205	0.5041
EE	25.00a	−96.60b	−35.80B	60.20a	−16.20b	22.00A	61.80a	−26.40b	17.70A	74.25a	4.25b	35.00A	90.20a	9.20b	49.70A	0.04	< 0.0001	< 0.0001	0.3009
NDF	41.92	34.25	38.08	12.40	23.95	18.17	23.90	49.47	36.68	33.07	39.96	36.51	43.44	47.48	45.46	0.004	0.0818	0.1174	0.2938
NFC	73.60	72.40	73.00	81.80	82.00	81.90	77.80	74.80	76.30	75.45	78.45	76.95	76.00	81.60	78.80	0.01	0.0821	0.7009	0.7843
Intestinal digestibility																			
CP	66.49b	70.76a	68.62	64.76b	68.24a	66.50	65.63b	67.74a	66.68	71.28b	74.46a	72.87	71.54b	72.77a	72.15	1.18	0.4782	0.0339	0.9401
EE	51.22b	88.76a	69.99	63.89b	83.50a	73.69	64.07b	79.77a	71.92	48.83b	84.35a	66.59	41.12b	80.99a	61.05	2.54	0.3231	< 0.0001	0.5195

CS—corn silage; SCIN—sugar cane *in natura*; SCS0%—untreated sugar cane silage; SCS0.4%—sugar cane silage treated with 0.4% calcium oxide; SCS0.8%—sugar cane silage treated with 0.8% calcium oxide; Treat—treatment; T × L = interaction treatment vs. location; SEM—standard error of the mean; Ret—reticulum; Om—omasum; DM—dry matter; OM—organic matter; CP—crude protein; EE—ether extract; NDF—neutral detergent fiber; NFC—non-fiber carbohydrates; TDN—total digestible nutrients. Capital letters compare diets on the same line and lowercase letters compare sampling locations on the same line.
Means followed by different lowercase letters in the same row or followed by different capital letters in the same column differ ($P < 0.05$).

expansion and rupture of cellulose molecules. However, in the present study, the addition of calcium oxide at levels of 0.4% and 0.8% was not found to be sufficient to have any beneficial effect on the intake and digestibility of the tested diets. These findings demonstrate the need to assess the true effectiveness of the treatment of sugar cane silage with this additive.

It can be inferred that NDFi plays a key role in controlling the intake of DM because the intake of DM was different between diets, but the intake of NDFi did not change.

Given that no difference between the two sites of digesta collection was observed, the digesta collected in the reticulum can be used in place of omasal digesta to estimate the ruminal digestibility of DM, OM, CP, NDF and NFC. However, the values of the ruminal digestion of NDF obtained from the reticular digesta in the SCIN and SCS0% diets were lower than those obtained from the omasal digesta. Furthermore, the values for the ruminal digestibility of EE determined from the reticular digesta were not consistent and were high and positive, indicating an absence of microbial lipid synthesis in the rumen.

The intestinal digestibility of EE was found to be higher in the omasal digesta than in the reticular digesta. Mathison et al. (1995) stated the importance of the role of the reticulum as the organ that propels the digesta through the reticulum–omasal orifice, but the mechanisms and factors that control this process are not well understood. The complexity of these mechanisms indicates that the reticular digesta may not represent the digesta that will actually leave the rumen, consequently compromising the results.

The use of calcium oxide did not improve the rate of ingestion as well as NDFpd ruminal poll (Table 2) for sugar cane silage components, which explains the similarity observed regarding the intake of sugar cane silage-based diets. However, calcium oxide probably reduces the loss of dry matter during sugar cane ensiling.

5. Conclusions

Considering all diet constituents, the omasum is the preferred sampling location. Due to the ease of collection, additional studies should be conducted with reticular digesta because numerous nutrients in ruminal digesta are similar to those in omasal digesta. Considering that DM, OM and NDF were similar between *in natura* sugar cane and sugar cane silages, ensiling is a promising alternative for sugar cane utilization *in natura*. However, none of these diets promotes nutrient intake similar to that by maize silage. Calcium oxide represents no benefits for improved nutrient intake, but probably reduces the loss of dry matter during sugar cane ensiling.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

This research project was supported by the FAPEMIG, CNPq and INCT of Ciência Animal, Brazil.

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