Nutritive value of maize and sorghum silages: fibre fraction degradation and rumen microbial density in buffalo cows

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ABSTRACT: Sorghum could be a potential substitute to maize in Mediterranean buffaloes feed in order to improve sustainability of buffalo-based agriculture, due to its reduced water and nitrogen requirements compared with maize, which is currently fed primarily. The aim of this study is to obtain information on rumen degradability of fibre fraction of maize and sorghum silages and to investigate the relationship between degradability and rumen microbial populations. As such four cannulated buffalo milking cows were fed *ad libitum* two different iso-energetic and iso-proteic diets based on maize silage (MS) and sorghum silage (SS). Based on plate counts, values of cellulolytic bacteria showed to be higher within the rumen of SS fed buffaloes compared to MS fed buffaloes (4.4×10^9 vs 1.9×10^9 cfu/ml, $P < 0.05$), on the contrary, those of xylanolytic bacteria (3.2×10^9 vs 1.3×10^9 cfu/ml, $P < 0.01$) were higher in MS possibly due to the different fibre degradability. Real-time PCR of total bacteria, *Fibrobacter succinogenes*, *Ruminococcus albus,* and *Ruminococcus flavefaciens* revealed no statistical difference in their 16S rDNA concentrations between diets. MS and SS were subsequently utilized for a degradability experiment. For this trial three cannulated Mediterranean dry buffalo cows were used (body weight 580 ± 8.5 kg). The MS was found to have an effective degradability of acid detergent fibre, hemicelluloses, and cellulose which were always lower than SS. Maize neutral detergent fibre degradability and slowly degradable fraction were significantly ($P < 0.01$) higher, on the contrary the immediately degradable fraction was found to be significantly ($P < 0.001$) lower compared with sorghum. The better sorghum relative feed value ($P < 0.001$) was related to the major content of fibre fraction compared to maize. As recommended by the IPCC Guidelines, Tier 2 was chosen to estimate the enteric CH_4 emission factor. The estimate of methane production is significantly lower in animals eating sorghum rather than maize (63.48 and 103.00 kg CH_4/head/year respectively, $P < 0.001$). In conclusion, as no difference was observed in animal weight gain and milk yield, rumen microbiota or degradability, it could be possible to substitute MS with SS in buffalo diet.

Keywords: ruminants; rumen microorganism; rumen degradability; forages

INTRODUCTION

Maize silage (MS) represents the main forage in the diets of dairy and buffalo cows (*Bubalus bubalis* L.), but shows some weak aspects in terms of qualitative and quantitative production: a high contamination by mycotoxins, maize parasites (e.g. *Diabrotica virgifera virgifera* and *Ostrinia nubilalis*), the increasing cost of irrigation, fertilization, and weed control which may limit the

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quality and quantity of productions. All tolerance limits of contamination are listed in the European Commission Regulation No. 165/2010 (European Commission, 2010). Compared to maize, sorghum has an increased thermal demand (seed germination occurs at 14°C, compared to 12°C of maize) and less water demand. For these reasons there is an interest in studying the possibility of totally or partially substituting MS with sorghum silage (SS). Indeed sorghum has long been used in animal feeding, particularly in dry and marginal areas (Barile et al., 2007), and MS and SS fed buffalo cows gave similar results in daily milk yield (kg) and milk fat and protein.

Dry matter intake (DMI) is critical to animal performance and cell-wall concentration, i.e. neutral detergent fibre (NDF) of forages is negatively associated with intake of forages because of its contribution to ruminal fill (Jung and Allen, 1995; National Research Council, 2001). In a review Oba and Allen (1999) stated that when *in situ* or *in vitro* NDF effective degradability (dNDF) increased by 1% in MS diets, DMI increased by 0.17 kg and milk yield by 0.25 kg in cows. Effective degradability (d) of NDF, acid detergent fibre (ADF), cellulose (C), and hemicelluloses (HC) are the most important feed characteristics to determine feed/ration value, due to the wide variation in NDF concentration and degradation among feeds (Huhtanen et al., 2006; Bossen et al., 2008).

Ruminant herbivores depend on microbial fermentation within the rumen to acquire energy from plant material. In particular buffaloes are able to utilize feed more efficiently than cattle and are more efficient in a number of other aspects, such as N-recycling, fibre degradation, fermentation, and intake and a higher number of cellulolytic bacteria (CB) (Wanapat et al., 1994; Puppoet et al., 2002). Moreover, propionate and butyrate concentrations were higher in buffalo compared to cattle (Wora-anu, 2006).

The digestion of plant material is a process involving strictly-anaerobic CB, protozoa (P), and fungi (F), with bacteria being the most abundant and diverse (Mackie, 1997). *Fibrobacter succinogenes, Ruminococcus flavefaciens,* and *Ruminococcus albus* are considered to be the main CB in the rumen (Denman and McSweeney, 2005; Shinkai and Kobayashi, 2007; Wanapat and Cherdthong, 2009). Nonetheless, recent metagenomic-based analysis has revealed the presence of novel ruminal cellulases, suggesting that other as yet uncultured ruminal bacteria are involved in ruminal plant cellulose degradation (Brulc et al., 2009; Duan et al., 2009).

Methane from agriculture arises primarily from enteric fermentation; therefore, ruminants are mainly responsible for enteric emissions of CH_4 (Kebreab et al., 2008). Sarubbi et al. (2010) estimated an average of CH_4 emission factor of 61 kg CH_4/head/year for buffalo cows and 47 kg CH_4/head/year for buffalo bull in the worldwide.

As the nutritive value of MS is related to its nutrient concentration and degradation characteristics, and because of the scarce knowledge about the digestion kinetics of the structural polysaccharides in the buffalo, the aim of this study was to obtain information on rumen degradability of fibre fraction of MS and SS and to investigate the relationship between degradability and the rumen microbial populations.

MATERIAL AND METHODS

Feed chemical analyses. MS (*Zeamais* – cultivar CV Kamil class FAO 400) and SS (*Sorghum vulgare* – Nicol-Pioneer + Trudan 8-NK) were utilized in the trial. The crops were seeded in May and the silages were harvested in September. Each forage was chopped and ensiled in two bunker silos for approximately 40 days without addition of lactic ferments. After ensiling, 3 representative samples of each silage were taken. The samples were pooled, dried at 65°C for 48 h, milled (1.1 mm screen) with 1090 Cemotec™ Sample Mill (Foss Tecator AB, Höganäs, Sweden), and analyzed (in triplicate) for dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fibre (CF) (methods 930.15, 942.05, 976.05, 954.02, and 978.10, respectively of the AOAC, 1998). Fibrous carbohydrates were fractionated using the Fibertec (VWR International LLC, Randor, USA) apparatus, NDF was determined in accordance with Van Soest et al. (1991) without α-amylase. ADF was determined in accordance with a standard method of AOAC (method 973.18), and was expressed inclusive of residual ash. Lignin (ADL) was determined by solubilization of cellulose with 720 g/kg sulphuric acid (Robertson and Van Soest, 1981) (Table 1).

As recommended by the IPCC Guidelines for National Greenhouse Gas Inventories (Eggleston et al., 2006), Tier 2 was chosen to estimate the enteric CH_4 emission factor. Relative forage value (RFV) and relative forage quality (RFQ) were

Table 1. Chemical composition of maize silage and sorghum silage (g/kg DM), diets (% on DM) and net energy used for *in situ* trial, GE, DE, and ME (MJ/kg DM); DE/GE and ME/GE (MJ/MJ)

Chemical composition	Maize silage	Sorghum silage
DM (g/kg)	281.0 ± 1.0	230.0 ± 0.0
CP (g/kg DM)	86.0 ± 1.0	79.4 ± 0.2
EE (g/kg DM)	34.1 ± 0.1	32.9 ± 0.2
Ash (g/kg DM)	87.9 ± 1.8	97.5 ± 1.5
CF (g/kg DM)	320.0 ± 2.0	351.0 ± 2.0
NDF (g/kg DM)	531.0 ± 4.0	630.0 ± 1.0
ADF (g/kg DM)	362.0 ± 4.0	407.0 ± 2.0
ADL (g/kg DM)	43.2 ± 0.5	36.1 ± 0.5
C (g/kg DM)	318.8 ± 3.6	370.9 ± 1.4
HC (g/kg DM)	169.0 ± 1.0	223.0 ± 2.0
GE (MJ/kg DM)	11.8 ± 0.7	14.5 ± 0.1
DE (MJ/kg DM)	13.4 ± 0.4	13.6 ± 0.1
ME (MJ/kg DM)	8.5 ± 0.1	8.2 ± 0.1
DE/GE (MJ/MJ)	1.1 ± 0.1	0.9 ± 0.0
ME/GE (MJ/MJ)	0.7 ± 0.0	0.6 ± 0.0
	diets with maize silage	diets with sorghum silage
DM (%)	15.9	16.1
CP (% DM)	15.8	15.5
CF (% DM)	21.1	21.4
NDF (% DM)	35.4	36.1
ADF (% DM)	26.5	23.5
EE (% DM)	4.3	3.8
Ash (% DM)	5.8	7.3
Starch (% DM)	23.5	21.3
Ca (% DM)	0.6	0.6
P (% DM)	0.4	0.4
MFU/kg DM	0.9	0.9

DM = dry matter, CP = crude protein, EE = ether extract, CF = crude fibre, NDF = neutral detergent fibre, ADF= acid detergent fibre, ADL = acid detergent lignin, HC =hemicelluloses (NDF-ADF), C = cellulose (ADF-ADL), GE = gross energy, DE = digestible energy, ME = metabolized energy, MFU = milk forage unit

calculated according to the National Research Council (2001). Mean, standard deviation, and minimum and maximum data of total digestible nutrient (TDN) and CH_4 estimated production in both silages are reported in Table 2.

Degradability trial. Three cannulated Mediterranean dry buffalo cows (body weight 580 ± 8.5 kg) were used in degradability trial. The buffaloes were fed diet (11 kg DM/day/head; MS (50%), ryegrass hay (40%), concentrate (10%)) in two equal meals at 09.00 h and 17.00 h, while fresh water was freely available. The ratio was calculated by assigning 0.72 milk forage units (MFU/kg DM), 10.5% of CP and 21% of CF, supplemented with vitamin-mineral mix. Samples used for *in situ* incubation were milled with 3 mm ground screen and approximately 7 g amount was inserted into dacron bags (approximately 15 mg/cm^2 of free bag area; porosity 40 μm) of 11 × 7 cm. Samples were incubated in the rumen in the morning before feeding for 0, 4, 8, 16, 24, 48, 72, and 120 h (two bags/incubation time/animal). After incubation, at each sampling time, the bags were immediately immersed in cold water and washed in a washing machine set to a cold cycle for about 15 min. The 0 h sample was washed in a washing machine, without rumen incubation, and using a cold cycle for approximately 15 min. After washing all bags were dried (48 h at 65°C) and weighed. Bag residues at each incubation time were pooled and NDF, ADF, HC, and C contents were determined. According to curve peeling method, potential degradability parameters were determined using the following models:

$$p = a + b(1 - e^{-ct})$$

where:
p = potential degradability
a = immediately degradable fraction
b = slowly degradable fraction
e = natural logarithm
c = degradation rate
t = incubation time

NDF, ADF, and HC degradation data at each incubation time were corrected for insoluble material washed from the bag by using a variant of an equation from Weisbjerg et al. (1990), as cited by Steinsig et al. (1994). Because NDF, ADF, and HC are insoluble, it was assumed that the truly soluble fraction was at 0 h wash, so the equation of Weisbjerg et al. (1990) was simplified and applied as follows:

$$K(t_i) = M(t_i) - P\,(1 - (M(t_i) - P/1 - P))$$

where:
$K(t_i)$ = corrected degradation at time t_i (g/kg)
$M(t_i)$ = uncorrected measured degradation at time t_i (g/kg)
P = insoluble fraction washed from the 0 h bag (total fraction washed from the 0 h bag (g/kg))

Table 2. Total digestible nutrient (TDN), intake potential, digestible dry matter (DM), relative feed value (RFV), forage quality (RFQ), and CH_4 production (kg/head/year)

	Maize silage			Sorghum silage			*P*-value
	mean	min	max	mean	min	max	
TDN	74.66 ± 3.27	70.1	77.8	59.04 ± 1.60	56.6	60.4	0.001
Intake potential (kg)	2.68 ± 0.24	2.35	2.86	1.94 ± 0.03	1.92	1.98	0.001
Digestible DM	65.17 ± 3.20	60.7	67.78	58.85 ± 1.23	57.2	60.1	0.001
RFV	18.90 ± 0.81	18.2	20.02	23.48 ± 0.78	22.4	24.1	0.001
RFQ	83.83 ± 1.92	81.6	86.79	71.21 ± 0.40	70.6	72	0.001
CH_4 (kg/head/year)	103.00 ± 10.28	82.2	117.8	63.48 ± 20.25	48.2	96.6	0.001

The data were fitted with the exponential model (Ørskov and McDonalds, 1979). The parameters from this model were used to calculate effective degradability of NDF, ADF, HC, and C according to the equation:

$$d = a + b\ (c/c + k)$$

where:
d = effective degradability
a, b, c = potential degradability parameters
k = ruminal passage rate (0.03/h)

Microbiological trial. Four cannulated lactating Mediterranean buffalo cows were fed *ad libitum* two different diets, similar in terms of energy and protein content (0.90 MFU/kg DM and 155 g CP/kg DM) and composed as follows (%): MS diet (MS 71.2, lucerne hay 9.3, concentrate 19.0) and SS diet (SS 60.9, lucerne hay 10.1, concentrate 28.5). The animals were fed once a day, for a 3-month period, according to cross-over design. Groups were homogeneous for the following parameters: days in milk (30 days), milk production (5.2 kg/head/day), body weight at the beginning of the trial (600 ± 10 kg).

Rumen samples (1 l) were collected from each animal 1 h before the morning feeding for three consecutive days, after two weeks of adaptation. The whole rumen fluid was used for pH, NH_3, volatile fatty acids (VFA) determination, and the protozoa (P) counts, performed in a Fuchs-Rosenthal chamber according to the Warner procedure (Warner, 1962). VFA (lactic, acetic, propionic, and butyric) were analyzed using high-performance liquid chromatography (HPLC) (Waters 2695 with 2487 Detector System) (Waters, Milford, USA) according to Lívian de Sáet et al. (2011) procedure.

Another aliquot was strained (3 layers of muslin) and treated with a homogenizer to detach the microorganisms from food particles, than diluted and incubated under anaerobic condition at 39°C (atmosphere 95% CO_2, 5% H_2) (Thermo Scientific, Denver, USA). Total viable bacteria liquid and solids media (TVBL and TVBS) and xylanolytic bacteria (XB) were grown in Leedle and Hespell medium (Leedle et al., 1982), CB in Hungate medium (Hungate, 1966), F in Joblin medium (Joblin, 1981). Liquid cultures were counted using the Most Probable Number procedure.

DNA was extracted from the pellets using GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, St. Louis, USA) according to manufacturer's guidelines. DNA concentration was quantified using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, USA). Q-PCR quantification of total bacteria content, and *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, was performed using a 7500 Real-Time PCR system (Applied Biosystems, Warrington, UK) as described by Huws et al. (2010). Q-PCR efficiency for all assays was 90–110%, except for the *R. flavefaciens* assay where we could only achieve efficiencies of 75–80%. Correlations of genomic DNA standards for all QPCRs were > 0.97.

Statistical analysis. ANOVA was carried out to evaluate significant differences between the two silages. The Pearson's correlation was used to evaluate the relationship among parameters.

Linear regressions were used to develop prediction equation for NDF and ADF degradability using chemical concentration of the tested substrates.

The microbiological data were analyzed using the General Linear Models procedure of SPSS (Version 12.0, 2003). Least Squares Means and pooled standard error of means were obtained.

The model used for experiment was:

$$Y_{ijkl} = m + A_i + b_j + C_k + (AB)_{ij} + (ABC)_{ijk} + e_{ijkl}$$

where:

m = means
A_i = species (i = 1, 2)
B_j = diets (j = 1, 2)
C_k = withdrawal (k = 1,..., 3)
$(AB)_{ij}$, $(ABC)_{ijk}$ = interactions
e_{ijkl} = error

All statistical methods of data evaluation were done using SPSS software (Version 12.0, 2003).

RESULTS AND DISCUSSION

The nutrient concentration of diets and chemical composition of silages are reported in Table 1. MS alone had higher DM and CP, but NDF, ADF, C, HC, and ash were higher in SS. Table 2 shows that MS has a better DM digestibility ($P < 0.001$), lower RFV values ($P < 0.001$), and higher RFQ ($P < 0.001$) than SS.

The positive relationship between RFV and RFQ in SS and MS is shown in Figure 1. RFV is used to compare forages for two important qualities – how well it will be consumed and how well it will be digested. This result is crucial to balance degradability and fermentability in the ratio formulation, in fact the higher RFV content was correlated with the higher content of fibre fractions. The RFQ index reflected the performance that can be expected in buffalo fed these forages, and the MS showed a better degradability.

Table 2 points out that CH_4 estimated production is significantly lower in SS ($P < 0.001$), resulting in a lower potential greenhouse gas (GHG) emissions.

MS showed effectively degradable fraction (dNDF, dADF, and dC) and slowly degradable fraction (bNDF, bADF, bC) always higher than SS (Table 3).

Table 3. Potential (a, b) and effectively degradable (d) (g/kg) of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose (C), and hemicelluloses (HC) in maize and sorghum silage

	Maize silage	Sorghum silage	*P*-value
dNDF	60.70	57.60	0.01
aNDF	16.62	27.93	0.001
bNDF	82.19	68.02	0.001
dADF	54.71	46.56	0.001
aADF	5.85	6.49	0.01
bADF	92.10	89.14	0.01
dC	52.65	44.64	0.001
aC	1.94	3.78	0.001
bC	95.97	91.71	0.01
dHC	73.42	78.86	0.01
aHC	38.52	65.69	0.001
bHC	61.35	31.71	0.001

d = effectively degradable fraction, a = immediately degradable fraction, b = slowly degradable fraction

The opposite trend is shown by immediately degradable fraction (a), these differences are always significant. The trend of the potential degradation parameters reported in Table 3 shows the increased solubility of the fibre degraded in the rumen (a) compared to the proportion degraded over time (b). This result is crucial to balance degradation and fermentability in the diets formulation.

The mean disappearance rates of NDF, ADF, C, and HC in the SS and MS are presented in Figures 2 and 3. At each time the MS presented potential ADF and C degradability higher than SS during the first 16 h of incubation. HC potential degradability is every time higher in SS in the first 16 h of incubation. The NDF differences between silages are statistically significant at 4, 48, and 72 h of incubation ($P < 0.01$).

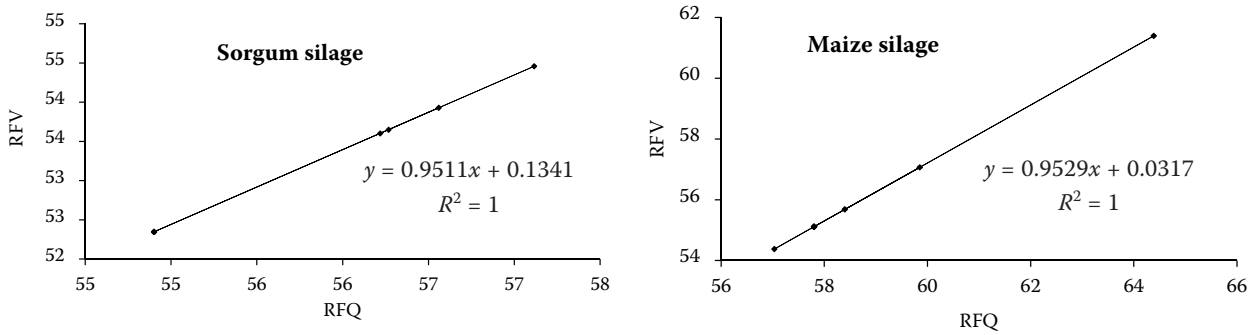

Figure 1. Relationship between relative feed value (RFV), forage quality (RFQ) in sorghum and maize silage

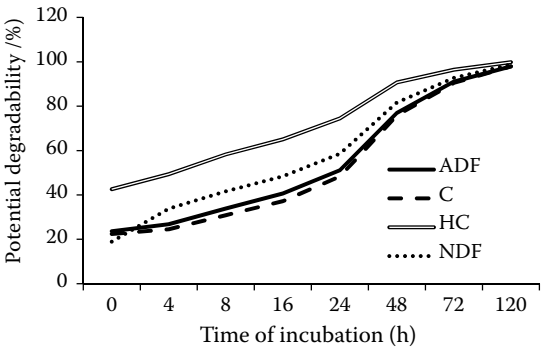

Figure 2. Mean disappearance rates of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose (C), and hemicelluloses (HC) in maize silage

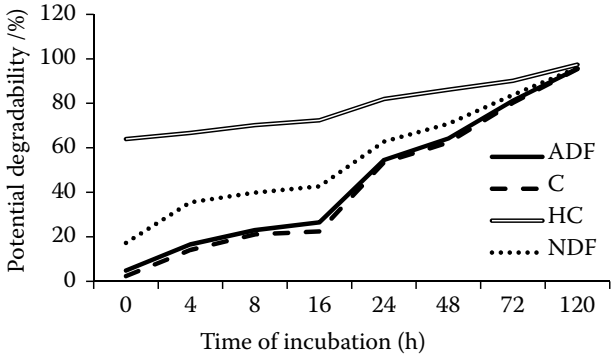

Figure 3. Mean disappearance rates of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose (C), and hemicelluloses (HC) in sorghum silage

Results of the coefficient of determination (R^2) between degradation parameters estimates and RFV scores are presented in Table 4. In MS the highly significant relationship between RFV and dNDF is justified because RFV is essentially the re-expression of NDF (Weiss, 2002). No significant correlation was found between RFV and ADF, HC and C degradability. Interesting appears to be the negative correlation between RFV and degradability of ADF and C in SS, that could be due to the definition of RFV, which does not take into account the component of cellulose in plant cell, as reported by Weiss (2002).

Table 5 shows that dNDF and dADF values were negatively related with all fibre fraction parameters. No significant correlation was observed between dHC and dC value and fibrous fraction, the table shows that the high degradability of HC reduces CP, CF, and NDF availability to livestock. Table 6 gives the correlation coefficients between the potential degradability (a, b) variables of NDF, ADF, HC, and C vs fibre nutrient characteristics. It shows that bNDF and bHC values were negatively related with all fibre fraction parameters except for ADL fraction. No significant correlation was observed between C value and fibrous fraction. The immediately degradable fraction (a) for NDF and HC was positively related with CF, NDF, ADF, HC, and C content and negatively related to ADL content.

Prediction equation for effective degradability. Linear regressions were used to develop prediction equation for ADF degradability using ash content of substrates tested. In general, the model presented was equal for each silage, prevision equation was significant ($P < 0.001$). The equation was the following:

$$dADF = -40.553 - 0.886\ (ash) \pm \varepsilon$$
$$(R^2 = 0.784 - R^{2Adj} = 0.763)$$

The prediction equations for other degradability parameters were not presented because no significant parameters were found.

Microbiological trial. As shown in Table 7, no significant differences were revealed in fungal density (expressed as cfu/ml). On the contrary, the CB values showed the tendency of being higher when buffaloes were fed the SS diet compared to MS diet (4.4×10^9 vs 1.9×10^9 cfu/ml, $P < 0.05$). Considering that the two diets had the same energy and protein content and the microflora had the same quantity of available nutrients, probably the CB were positively affected by a higher immediately degradable fibre fractionin SS diet as

Table 4. Coefficient of determination (R^2) of effectively degradable fraction (d) of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicelluloses (HC), and cellulose (C) and their relative feed value

Degradability parameters	Maize silage		Sorghum silage	
	R^2	*P*-value	R^2	*P*-value
dNDF	0.998	0.001	0.997	0.0001
dADF	0.389	ns	–0.145	ns
dHC	0.635	ns	0.532	ns
dC	0.142	ns	–0.137	ns

d = effectively degradable fraction, ns = not significant

Table 5. Correlation between effectively degradable fraction (d) of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicelluloses (HC), and cellulose (C) vs chemical composition of diets

	CP	CF	NDF	ADF	Hemicellulose	Cellulose	*P*-value
dNDF	0.649	0.649	–0.649	–0.649	–0.649	–0.649	0.01
dADF	0.886	0.886	0.886	0.886	0.886	0.886	0.01
dHC	–0.075	–0.156	–0.252	0.178	–0.178	–0.382	ns
dC	0.088	0.215	0.312	–0.222	0.222	0.473	ns

CP = crude protein, CF = crude fibre, ns = not significant

Table 6. Correlation coefficients between degradability variables (a, b) of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicelluloses (HC), and cellulose (C) vs fibre nutrient characteristics of diets

	CF	NDF	ADF	ADL	Hemicellulose	Cellulose	*P*-value
aNDF	0.734	0.734	0.734	–0.734	0.734	0.734	0.01
bNDF	–0.699	–0.699	–0.699	0.699	–0.699	–0.699	0.01
aADF	–0.417	–0.417	–0.417	0.417	–0.417	–0.417	ns
bADF	0.187	0.187	0.187	–0.187	0.187	0.187	ns
aHC	0.921	0.921	0.921	–0.921	0.921	0.921	0.01
bHC	–0.893	–0.893	–0.893	0.893	–0.893	–0.893	0.01
aC	–0.413	–0.413	–0.413	0.413	–0.413	–0.413	ns
bC	–0.410	–0.410	–0.410	0.410	–0.410	–0.410	ns

ADL = lignin, CF = crude fibre, a = immediately degradable fraction, b = slowly degradable fraction, ns = not significant

shown in Table 3. This confirms the published data by Puppo et al. (1999, 2002) when the buffaloes were fed more digestible fibre. XB were higher in MS and/or SS diet (3.2×10^9vs 1.3×10^9 cfu/ml, $P < 0.01$) and this could be ascribed to the higher slowly degradable fibre fraction (Table 3).

The pH values and NH_3 values (NH_3/total nitrogen) did not show any differences and among

Table 7. Microbial counts (cfu/ml), NH_3 (NH_3/total nitrogen), volatile fatty acids (VFA) (mg/100 mg), and pH values according to diets

	MS	SS	*P*-value
pH	6.6 ± 0.21	6.7 ± 0.27	ns
NH_3	3.62 ± 1.56	3.65 ± 0.81	ns
Lactic acid	2.37 ± 1.14	2.09 ± 0.50	ns
Acetic acid	0.67 ± 0.39	0.60 ± 0.21	ns
Propionic acid	0.19 ± 0.13	0.09 ± 0.06	0.05
Butyric acid	0.16 ± 0.15	0.09 ± 0.05	ns
P	$3.3 \times 10^8 \pm 0.32 \times 10^8$	$2.0 \times 10^8 \pm 0.39 \times 10^8$	ns
F	$4.4 \times 10^5 \pm 0.11 \times 10^5$	$4.1 \times 10^5 \pm 0.21 \times 10^5$	ns
TVBL	$1.9 \times 10^{11} \pm 0.02 \times 10^{11}$	$3.2 \times 10^{11} \pm 0.09 \times 10^{11}$	ns
CB	$1.9 \times 10^9 \pm 0.45 \times 10^9$	$4.4 \times 10^9 \pm 0.40 \times 10^9$	0.05
TVBS	$1.4 \times 10^{10} \pm 0.10 \times 10^{10}$	$6.3 \times 10^{10} \pm 0.19 \times 10^{10}$	0.05
XB	$3.2 \times 10^9 \pm 0.84 \times 10^9$	$1.3 \times 10^9 \pm 0.67 \times 10^9$	0.01

MS = maize silage, SS = sorghum silage, P = protozoa, F = fungi, TVBL = total viable bacteria liquid, TVBS = total viable bacteria solid, CB = cellulolytic bacteria, XB = xylanolytic bacteria, ns = not significant

Table 8. Effect of diets on bacterial rumen populations (data are shown as $\log_{10}$ pg/g DM)

	MS	SS	SE	*P*-value
Total bacteria	8.77	8.51	0.26	ns
Fibrobacter succinogenes	5.90	5.54	1.58	ns
Ruminococcus albus	5.26	4.95	0.50	ns
Ruminococcus flavefaciens	5.87	5.53	0.50	ns

MS = maize silage, SS = sorghum silage, SE = standard error deviation, ns = not significant, DM = dry matter

the VFA, propionate only was significantly higher ($P < 0.05$) in MS diet (Table 7).

In Table 8, the effect of diets on bacterial rumen population is reported. Total bacteria, *R. albus*, *R. flavefaciens* were similar on both diets. This lack of correlation of cellulolytic population on a diet that had higher NDF and ADF content was also noted within steers by Huws et al. (2010). Metagenomic-based analysis suggests that other as yet uncultured ruminal bacteria are involved in ruminal plant cellulose degradation (Brulc et al., 2009; Duan et al., 2009).

We could not observe that *F. succinogenes* is always the most abundant among the CB in rumen as reported in literature (Koike and Kobayashi, 2001; Wanapat and Cherdthong, 2009), in fact this is true only in MS. The slightly higher (even if not statistically significant) quantity of the three together (*F. succinogenes, R. albus,* and *R. flavefaciens*) (MS 17.03 and SS 16.02 $\log_{10}$ pg/g DM, $P <$ non-significant) could be due to the increase of NDF disappearance as reported by Koike et al. (2003).

Table 9 shows the correlation between NDF degradability and rumen microorganisms. The intermediate degradability fraction (a) was negatively correlated with TVBL ($R = -0.343$, $P < 0.05$). The degradation rate (c) was negatively correlated with XB content ($R = -0.392$, $P < 0.05$) and positively affected protozoa growth ($R = 0.421$, $P < 0.05$). A similar result has been reported by Puppo et al. (2002) in buffalo, where a better digestion of CP in a diet with high content of structural carbohydrates was pointed out. No statistical difference was observed between the two groups both in animal daily weight gain (MS 0.12 vs SS 0.04 kg/day) and milk yield (MS 7.5 vs SS 7.4 kg/head/day) and quality.

Table 9. Correlation coefficients between rumen microorganisms and potential degradability parameters of neutral detergent fibre (dNDF) in maize silage

	a	b	c	dNDF	*P*-value
TVBL	–0.343	0.324	0.302	0.042	0.05
CB	–0.063	0.063	0.034	–0.027	ns
TVBS	0.145	–0.107	–0.302	–0.034	0.05
XB	0.308	–0.263	–0.392	–0.277	0.05
P	–0.307	0.293	0.421	0.322	0.05

P = protozoa, TVBL = total viable bacteria liquid, TVBS = total viable bacteria solid, CB = cellulolytic bacteria, XB = xylanolytic bacteria, a = immediately degradable fraction, b = slowly degradable fraction, c = degradation rate, ns = not significant

CONCLUSION

This trial provides new information both on the rumen degradability of MS and SS and on the nutritive quality of sorghum, a substitute feed to maize. The results also showed the possibility of estimating the ADF degradability using the prevision equation.

As no differences were reported either on microbial environment, total bacterial populations, or production parameters (milk yield and quality), it could be possible to substitute MS with SS in the buffaloes' diet, particularly in marginal and arid areas.

Furthemore, CH_4 estimated production, significantly lower in sorghum diet, could result in a lower potential GHG emissions.

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