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In vitro degradability, gas production, and energy value of different hybrids of sorghum after storage in mini-silos

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IN VITRO DEGRADABILITY, GAS PRODUCTION, AND ENERGY VALUE OF DIFFERENT HYBRIDS OF SORGHUM AFTER STORAGE IN MINI-SILOS

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Abstract

This experiment compared silages obtained from 3 hybrids of sorghum grown on 2 farms of the Po Valley (one irrigated and one not), in terms of *in vitro* degradability, gas production (GP), and energy value. Hybrids (forage, sweet or grain genotypes) were sown in experimental plots (3 plots×3 hybrids), harvested at late-milk stage of maturity, and ensiled into mini-silos (3 silos×3 hybrids) for 60 d. After ensiling, silages were analyzed for composition and fermentation profile. Two incubations (at 48 h) were carried out to measure NDF degradability (NDFd), GP, and the metabolizable energy (ME) content of silages. Data of silage composition were submitted to ANOVA, considering farm (F), hybrid (H), and F × H interaction as variation sources. Incubation (run) was also considered as a fixed effect in the statistical model for the parameters obtained by *in vitro* incubation (NDFd, GP, and energy content). On the irrigated farm (Farm 2), the DM contents of silages were higher than those of the non-irrigated one ($P<0.001$) and the fermentation profile was more favorable. Values of GP at 24 and 48 h and ME content were higher ($P<0.05$) for silages of Farm 2 in comparison with Farm 1. Within hybrids, the grain sorghum revealed the greatest DM content whereas the forage sorghum, as expected, was the richest in fibrous fraction content, followed by the sweet and grain genotypes ($P<0.001$). Consequently, values of GP were significantly ($P<0.01$) influenced by hybrid (167, 200, 215 ml/g DM and 229, 257, 267 ml/g DM for forage, sweet and grain genotypes after 24 and 48 h of incubation, resp.). The F × H interaction was significant for all considered parameters excluding DM, lignin, ash, pH, and *in vitro* parameters. On the two farms, in general, forage and grain genotypes were largely different, whereas the sweet sorghum was quite similar to the forage in one case or grain in the other. Results of this experiment highlight the large variability of the nutritional values of sorghum hybrids grown in different conditions.

Keywords: Sorghum hybrids; Sorghum silage; *In vitro* degradability; *In vitro* gas production

Introduction

Silages obtained from sorghums belonging to conventional forage and grain genotypes were found to be valid feed sources for dairy cows (Dann et al., 2008; Colombini et al., 2012). In the last years, the potential of sorghum silage as ruminant feed has been evaluated also in Europe. Results would suggest that the inclusion of such feed ingredient in dairy cow diets should be carefully considered, as partial replacement, i.e., to corn silage (Colombini et al., 2010, 2012; Śliwiński et al., 2012). Sweet sorghum represents a particular cultivar with a high content of sugars (70-80% sucrose) and, to date, it has mostly been used in energy plant for ethanol and biofuel production. However, for its specific chemical profile, some seed companies have been promoting sweet sorghum as a possible crop for silage production and ruminant feeding. Over the last few years *in vitro* gas production (GP) technique has been largely adopted to evaluate fermentation of ruminant feeds, because it is a fast and cost-effective analysis (Rymer et al., 2005). To date, only the study of Di Marco et al. (2009) has explored the fermentative properties of sweet sorghum silage, when incubated *in vitro* with rumen fluid, in comparison with forage and grain genotypes. Thus, this research is aimed at comparing *in vitro* degradability, GP, and energy value of silages obtained from forage, sweet, and grain sorghum grown in two farms located in the Po Valley (Northern Italy).

Material and methods

Three hybrids of *Sorghum vulgare* spp. were used: a forage sorghum (Bulldozer), promoted for its high biomass yield and traded by KWS Italia Spa (Monselice, Padova, Italy), a sweet sorghum (Surgo) and a grain sorghum (Favorite), both traded by SIVAM Spa (Casalpusterlengo, Lodi, Italy). Plants were grown in two pilot farms of the Veneto Agricoltura Agency, one (Farm 1) located in the province of Venice (Vallevecchia, latitude 45.6°N, longitude 12.9°E; 0 m above sea level) and one (Farm 2) located in the province of Rovigo (Ceregnano, latitude 45.0°N, longitude 11.9°E; 5 m above sea level). The farms were involved in a project aiming to evaluate quality of silages obtained from different genotypes of sorghum. In each farm, sorghums were sown in nine experimental plots (three plots per each hybrid) with an area of 0.2 ha each. Sowing took place in the first ten days of

June for all genotypes. No fertilizers were applied; urea (100 kg/ha) and herbicides were distributed at post-emergence phase. Irrigation of plants occurred only in Farm 2 (on July 2), as the Farm 1 is not equipped with an irrigation system. Sorghums were harvested on September 18, 2013 in Farm 1 and on September 12, 2013 in Farm 2, in order to collect from both sites plants at a late-milk stage of maturity. The chemical composition of fresh forages was the following (expressed as mean value of the two farms): 24.6, 27.3, and 33.1% DM; 5.0% CP, 60.5% NDF, 6.1% starch, 6.1% ash, for the forage sorghum (Bulldozer); DM, 5.8% CP, 58.5% NDF, 9.2% starch, 6.2% ash, for the sweet sorghum (Surgo); % DM, 8.1% CP, 55.5% NDF, 21.0% starch, 6.6% ash, for the grain sorghum (Favorite). After harvest, three aliquots of chopped forage (10 kg each) were prepared for each hybrid, as a representative sample of the three experimental plots, homogeneously mixed, and mechanically compacted into nine laboratory mini-silos (3 silos×3 hybrids) with 20 l capacity, using a press equipped with a manometer and a hydraulic cylinder generating a compressive force of 1.2 atm/cm^2. The mini-silos were hermetically closed and stored for 60 d at 24 ± 3°C. On opening the mini-silos, the upper layer (10-15 cm) of silage was discarded, to limit risk of taking samples with anomalous fermentation. After that, two aliquots (about 1.5 kg each) were prepared for each sorghum silage, as a representative sample of the three mini-silos. The same protocol was followed on both farms. The first aliquot of each silage was sent to the laboratories of ARAV (Breeders Association of Veneto Region, Padova, Italy) to assay proximate composition, pH, ammonia N content, and fermentation acid profile. Proximate analysis was conducted in triplicate according to AOAC (2012). The NDF fraction, inclusive of insoluble ash, was measured with Ankom^{220} Fibre Analyzer (Ankom Technology, NY, USA). Ammonia N content and pH were determined by a potentiometer equipped with a specific electrode (pH meter BASIC 20, Crison Instruments, Alella, Spain). Fermentation acids were measured using a Thermo Finnigan Spectra System AS3000 auto-sampler (Thermo Electron Corporation, Waltham, MA, USA), equipped with an H_2SO_4 0.0025 N Bio-Rad HPX-87H column (Bio-Rad Laboratories, Richmond, CA, USA). The second aliquot of each silage was sent to the laboratories of the University of Padova. Once in the

79 laboratories, samples were dried in a forced-air oven at 60°C for 48 h, to determine DM content,
80 and ground to 1-mm. Eight subsamples were prepared for each hybrid × farm combination and used
81 for *in vitro* tests. Fermentations were conducted with Ankom^{RF} gas production (GP) system
82 (Ankom Technology, NY, USA). This system is a kit of bottles (310 ml) equipped with a pressure
83 detector and wireless connection to a PC. Each bottle was filled with feed sample (0.500±0.0010 g),
84 25 ml of rumen fluid, and 50 ml of buffer solution (ratio 1:2). Bottles were incubated at 39 ± 0.4°C
85 for 48 h and vented at 3.4 kPa, to avoid overpressure conditions (Cattani et al., 2014). Two
86 incubations were repeated in 2 successive weeks, and the following experimental design was
87 applied: 3 hybrids×2 farms×4 replicates, plus 4 blanks (bottles without feed sample), giving a total
88 of 28 bottles incubated in each of the two incubations. At the end of each incubation run,
89 fermentation fluids were filtered into weighed crucibles (Robu Glasfilter-Geräte GMBH, Hattert,
90 Germany) and treated with a heat stable amylase, but without sodium sulphite, to assay residual
91 NDF, using a Fibertech Analyzer (VELP Scientifica, Milan, Italy). Rumen fluid was collected by an
92 esophageal probe, as detailed by Tagliapietra et al. (2012), from three intact dry Holstein-Friesian
93 cows fed hay *ad libitum* and 2.5 kg/d of concentrates. Buffer solution was prepared according to
94 Menke and Steingass (1988). The degradability of NDF (NDFd) and of true DM (TDMd) were
95 calculated as follows:
96 $NDFd\ (\%\ NDF) = [(NDF_{feed} - NDF_{res})/NDF_{feed}] \times 100$
97 where NDF_{feed} is the NDF content (g/kg DM) of feed incubated; NDF_{res} is the amount (g/kg DM) of
98 residual NDF
99 $TDMd\ (\%\ DM) = [(DM_{feed} - NDF_{res})/ DM_{feed}] \times 100$
100 where DM_{feed} is the DM content (g/kg) of feed incubated
101 Metabolizable energy (ME) content of silages was computed from chemical composition and NDFd
102 measured at 48 h (NRC, 2001; ME_{NRC}) or GP measured at 24 h of incubation (Menke and
103 Steingass, 1988; ME_{Menke}). The two equations were the following:

104 ME_{NRC} (MJ/kg DM) = − 0.45 ×4.184 + 1.01×DE

105 where DE is the digestible energy:

106 DE (MJ/kg DM) = [(NDFd/1000) ×4.2 + (tdNFC/1000) ×4.2 + (tdCP/1000) ×5.6 + (tdFA/1000)

107 ×9.5 – 0.3] × 4.184

108 where NDFd is the NDF degradability (g/kg NDF) measured at 48 h; tdNFC, tdCP and tdFA are the

109 estimated true digestible contents of non-fibre carbohydrates, CP and EE (g/kg DM) calculated

110 using the equations proposed by NRC (2001) (i.e., Eqs. 2–4a to 2–4e).

111 ME_{Menke} (MJ/kg DM) = 2.20 + 0.1357×$GP24_{200}$ + 0.0057×CP + 0.0002859×EE^2

112 where $GP24_{200}$ is the gas production (ml) measured at 24 h and referred to 200 mg of feed sample;

113 CP = crude protein content (g/kg DM); EE = ether extract content (g/kg DM)

114 *Statistical analysis*

115 Data of silage composition (proximate analysis, pH, fermentation acid profile, ammonia N) were

116 subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS

117 (SAS Institute Inc., Cary, NC, USA release 9.1). The statistical model considered effects of farm (2

118 levels: Farm 1 and Farm 2), hybrid (3 levels: Bulldozer, Surgo, and Favorite), and interaction

119 between farm and hybrid (F × H) as sources of variation. Other data (*in vitro* degradability, GP, and

120 energy content of silages) were analyzed using a model that considered effects of farm, hybrid, F ×

121 H interaction, and, in addition, incubation run (2 levels: incubation 1 and incubation 2) as sources of

122 variation.

123 **Results**

124 The DM content of silages was on average greater in Farm 2 compared to Farm 1 (29.0 vs. 25.5%,

125 respectively; P<0.001; Table 1). The proximate composition of silages reflected the plant genotype.

126 The forage sorghum had the greatest NDF, ADF, and ADL contents (P<0.001). On the other hand,

127 the grain genotype showed the lowest fiber fraction, especially in Farm 1, and the highest starch

128 content (P<0.001). As regards starch, the sweet genotype showed, on average, the lowest content in

129 Farm 1 and intermediate values in Farm 2. Starch content of the sweet sorghum was, on average,
130 three times greater on Farm 2 than on Farm 1 (13.2 vs. 4.4% starch in Farm 2 and Farm 1,
131 respectively). Final pH of silages was affected by farm, and hybrid ($P<0.001$; Table 2). In all silages
132 lactate was the prevalent fermentation acid (on average 83.1% total fatty acids), followed by acetate
133 (on average 16.7% total fatty acids); propionate was present only in traces and n-butyrate was never
134 detectable by the GC. Total production of fermentation acids was influenced by hybrid ($P<0.001$),
135 proving consistently lower for the forage genotype; in Farm 1 the sweet sorghum showed a lower
136 acid production compared to the grain genotype, whereas the opposite tendency was observed in
137 Farm 2 ($P<0.001$). The ratio between ammonia N and total N ranged from 2.97, for the sweet
138 genotype of Farm 2, to 6.54% for the grain genotype of Farm 1. Values of NDFd were not
139 influenced by hybrid and farm, and ranged from 50.2 to 57.3%, for the grain and the forage
140 sorghums grown in Farm 1 (Table 3). Compared to the other two hybrids, the sweet sorghum
141 revealed an intermediate extent of NDF degradability in the Farm 1 (NDFd=54.5%) and the lowest
142 value in the Farm 2 (NDFd=51.7%). Irrespective of the farm, the grain genotype showed the
143 greatest values of TDMd, whereas the lowest *in vitro* "true" DM degradability was found for the
144 forage genotype. As observed for NDFd, the sweet sorghum exhibited intermediate values of
145 TDMd with respect to other hybrids. As regards the sorghums of Farm 1, the grain genotype
146 showed the greatest values of *in vitro* GP ($P<0.001$ and $P<0.05$, at 24 and 48 h, respectively); no
147 differences were found between the other two hybrids (the forage and the sweet), neither at 24 h nor
148 at 48 h. A different ranking emerged for samples belonging to Farm 2, as the forage sorghum
149 always had the lowest *in vitro* GP ($P<0.001$ and $P<0.05$, at 24 and 48 h of incubation, respectively),
150 whereas the sweet sorghum showed an *in vitro* GP comparable to that of the grain genotype. In
151 terms of energy content the sweet sorghum tended to be more similar to the forage genotype in the
152 Farm 1 and to the grain genotype in the Farm 2. Values of ME_{NRC} ranged from 8.9 (for the sweet
153 genotype of Farm 1 and the forage genotype of Farm 2) to 10.1 MJ/kg DM (for the grain genotype
154 of Farm 2). Values of ME_{Menke} were on average lower than those calculated using NRC (2001)

155 approach and ranged from 7.0 to 8.9 MJ/kg DM for the forage and the grain genotypes of Farm 1,
156 respectively.

157 **Discussion**

158 Results of this study provide evidence that silages obtained from different sorghum hybrids differed
159 in terms of chemical composition, fermentation profile and nutritional value. In addition, the
160 cultivation site (farm) exerted a notable effect on silage characteristics. The DM content was largely
161 affected by hybrid and farm. Firstly, the genotype could have exerted an effect, as observed by
162 others (Pesce et al., 2000; Bolsen et al., 2003). Secondly, pedological characteristics of
163 experimental plots could have influenced DM accumulation in sorghum plants. More precisely,
164 soils belonging to Farm 1 were characterized, on average, by a lower OM, nitrogen, and mineral
165 contents (i.e. phosphorus and potassium) compared to those of Farm 2. Thirdly, an effect also could
166 be attributed to irrigation, which occurred only on Farm 2, where silages showed a greater DM
167 content. Sorghum is known to be a drought resistant plant (Sanchez et al., 2002); however, some
168 authors (Carmi et al., 2006) found that plants responded positively to irrigation, with an increment
169 of DM accumulation. Chemical composition of silages reflected substantially the hybrid genotype,
170 with a greater NDF content for the forage sorghum and a greater starch content for the grain one.
171 Up to now, data concerning chemical composition of sweet sorghum genotypes are scarce.
172 However, on the basis of our results, it could be speculated that irrigation promoted grain filling in
173 plants of the sweet sorghum grown in Farm 2, which showed a starch content three times greater
174 than the plants cultivated in Farm 1, where irrigation did not occur. In line with our expectations,
175 chemical differences led to different fermentation patterns during the ensiling process. However,
176 good visual appearance, colour and odour of silages seemed to indicate a proper preservation. In
177 support of that, pH values of silages were included in the expected range (3.48-4.50) reported by
178 Gallardo and Gagiotti (2004). Likewise, the ratio between ammonia N and total N was always
179 under the threshold of 7, which indicates a correct preservation of silages (Romero, 2004).
180 Moreover, fermentation acid profile, dominated by lactate and acetate, was an index of proper

ensiling into the mini-silos. Absence of significant effects due to the incubation run proves that the *in vitro* GP system used in this study has a satisfactory repeatability. The three sorghum genotypes showed different values of *in vitro* NDFd, and this confirmed data obtained *in vivo*, *in situ*, and *in vitro* by Di Marco et al. (2009). In line with previous findings (Pesce et al., 2000; Bolsen et al., 2003), the grain genotype showed the greatest values of TDMd and GP, as a result of greater starch content, whereas the forage sorghum showed the lowest values, as the fibrous fraction probably had a greater incidence on total DM degradability. In general, the sweet sorghum grown in Farm 1 had chemical characteristics and *in vitro* fermentative properties which were intermediate compared to the other two hybrids. However, the sweet sorghum seemed to be closer to the forage genotype in terms of DM and starch contents, *in vitro* GP, and energy value. Differently, the sweet sorghum grown in Farm 2 tended to be more similar to the grain genotype, especially in terms of *in vitro* fermentation properties and energy value.

The results of the present study would suggest that the cultivation and subsequent utilization of sorghum silages in ruminant feeding must necessarily consider the main peculiarities of each hybrid cultivated under different conditions. After ensiling, the sweet sorghum exhibited chemical characteristics and fermentative properties similar to those of the grain genotype, especially when plants were grown in irrigated fields. On this basis, silages obtained from sweet sorghum could be included in ruminant diets as total or partial replacement of corn silage, depending on the energy requirements of the animals. However, preliminary results presented in this paper should be validated *in vivo*.

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253

254 Table 1. Chemical composition (% DM) of three sorghum silages harvested in the two farms

	DM	Ether extract	CP	NDF	ADF	ADL	Ash	Starch
Farm 1								
Forage	22.3^{C}	2.0^{BC}	8.2^{BC}	70.1^{A}	41.9^{A}	4.6^{AB}	6.9^{AB}	5.8^{C}
Sweet	22.8^{C}	2.1^{BC}	9.4^{AB}	62.1^{B}	34.7^{B}	3.7^{BC}	7.6^{A}	4.4^{C}
Grain	31.3^{A}	3.3^{A}	10.2^{A}	49.3^{D}	27.4^{D}	2.9^{C}	7.5^{A}	15.8^{B}
Farm 2								
Forage	26.7^{B}	2.1^{BC}	8.2^{BC}	72.0^{A}	42.3^{A}	5.2^{A}	5.6^{C}	4.6^{C}
Sweet	26.6^{B}	2.4^{B}	8.7^{BC}	57.2^{BC}	32.8^{BC}	4.4^{AB}	6.4^{BC}	13.2^{B}
Grain	33.6^{A}	1.6^{C}	7.8^{C}	54.9^{C}	30.3^{CD}	3.9^{AB}	6.5^{BC}	20.0^{A}
SEM[1]	0.73	0.18	0.32	1.00	0.62	0.26	0.25	0.89
Farm (F)	***	*	***	ns	ns	***	***	***
Hybrid (H)	***	Ns	*	***	***	***	**	***
F×H	ns	***	**	***	**	ns	ns	***

255 Contrast significance is indicated ns=non-significant; *P≤0.05; **P<0.01; ***P<0.001

256 A, B, C, D – values in columns with different letters differ significantly (P≤0.01).

257 [1]SEM = standard error of the mean

Table 2. Silage pH, total production of fermentation acids (FA; g/kg as fed), proportion of acetate and lactate (% total FA), and proportion of ammonia N on total N ($N\text{-}NH_3/N$; expressed as percentage) of three sorghum silages harvested in the two farms

	pH	Total FA	Acetate	Lactate	$N\text{-}NH_3/N$
Farm 1					
Forage	3.97^{A}	14.4^{B}	19.3^{AB}	80.7^{DE}	3.97^{B}
Sweet	3.89^{AB}	15.6^{B}	20.4^{A}	79.5^{E}	5.35^{A}
Grain	3.95^{A}	18.2^{A}	17.5^{BC}	82.4^{CD}	6.54^{A}
Farm 2					
Forage	3.74^{CD}	14.8^{B}	13.5^{D}	86.3^{A}	3.46^{B}
Sweet	3.62^{D}	17.9^{A}	14.1^{D}	85.6^{AB}	2.97^{B}
Grain	3.81^{BC}	17.3^{A}	15.6^{CD}	84.0^{BC}	3.64^{B}
[1]SEM	0.032	0.34	0.56	0.56	0.317
Farm (F)	***	Ns	***	***	***
Hybrid (H)	***	***	ns	ns	***
F×H	ns	***	**	***	**

Contrast significance is indicated ns=non-significant; *P≤0.05; **P<0.01; ***P<0.001

A, B, C, D, E – values in columns with different letters differ significantly (P≤0.01).

[1]SEM = standard error of the mean

Table 3. *In vitro* degradability of NDF (NDFd, %) and of true dry matter (TDMd, %), *in vitro* gas production (ml/g DM), and metabolizable energy content (MJ/kg DM), calculated according to NRC (2001; ME_{NRC}) or to Menke and Steingass (1988; ME_{Menke}), of three sorghum silages harvested in the two farms

	NDFd	TDMd	Gas production		Energy value	
			24 h	48 h	ME_{NRC}	ME_{Menke}
Farm 1						
Forage	57.3^{a}	69.6^{bc}	156^{B}	220^{B}	9.1^{ab}	7.0^{C}
Sweet	54.5^{abc}	71.9^{abc}	181^{B}	236^{B}	8.9^{b}	7.8^{BC}
Grain	50.2^{c}	75.5^{a}	214^{A}	261^{A}	9.7^{ab}	8.9^{A}
Farm 2						
Forage	52.0^{bc}	68.5^{c}	177^{B}	237^{B}	8.9^{b}	8.0^{AB}
Sweet	51.7^{bc}	72.3^{ab}	219^{A}	278^{A}	9.6^{ab}	8.8^{A}
Grain	55.7^{ab}	75.9^{a}	216^{A}	273^{A}	10.1^{a}	8.6^{AB}
SEM	2.13	1.41	9.4	10.3	0.29	0.27
Incubation						
1	51.3	71.9	192	251	9.3	8.2
2	54.4	72.7	195	251	9.5	8.2
SEM	1.18	0.83	5.6	6.1	0.16	0.16
Farm (F)	ns	ns	*	*	ns	*
Hybrid (H)	ns	*	***	**	*	**
F × H	*	ns	ns	ns	ns	*
Incubation	ns	ns	ns	ns	ns	Ns

Contrast significance is indicated ns=non-significant; *P≤0.05; **P<0.01; ***P<0.001
a, b, c – values in columns with different letters differ significantly (P≤0.05).
A, B, C – as above for P≤0.01.
[1]SEM = standard error of the mean