### ANNALS OF ANIMAL SCIENCE ISSN: 2300-8733, <u>http://www.degruyter.com/view/j/aoas</u>

## **ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT:**

### In vitro degradability, gas production, and energy value of different hybrids of sorghum after storage in mini-silos DOI: 10.1515/aoas-2015-0082

Mirko Cattani<sup>1</sup>, Alberto Sartori<sup>2</sup>, Valerio Bondesan<sup>2</sup>, Lucia Bailoni<sup>1</sup>

<sup>1</sup>Department of Comparative Biomedicine and Food Science, University of Padova, Viale

dell'Università, 16, 35020 Legnaro (Padova), Italy

<sup>2</sup>Veneto Agricoltura Agency, Viale dell'Università, 14, 35020 Legnaro (Padova), Italy

♦Corresponding author: mirko.cattani@unipd.it

Received date: 30 July 2015 Accepted date: 24 November 2015

**To cite this article**: (2015). Cattani M., Sartori A., Bondesan V., Bailoni L. (2015). In vitro degradability, gas production, and energy value of different hybrids of sorghum after storage in mini-silos, Annals of Animal Science, DOI: 10.1515/aoas-2015-0082

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

Mirko Cattani<sup>1</sup>, Alberto Sartori<sup>2</sup>, Valerio Bondesan<sup>2</sup>, Lucia Bailoni<sup>1</sup>

<sup>1</sup>Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università, 16, 35020 Legnaro (Padova), Italy

<sup>2</sup>Veneto Agricoltura Agency, Viale dell'Università, 14, 35020 Legnaro (Padova), Italy Corresponding author: <u>mirko.cattani@unipd.it</u>

Abbreviated title: Nutritional value of sorghum silages

Work financed from Veneto Agricoltura Agency

Project title "Alternative crops rotation to contain diffusion of *Diabrotica virgifera virgifera* LeConte"

#### 1 Abstract

2 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 3 energy value. Hybrids (forage, sweet or grain genotypes) were sown in experimental plots (3 4 plots×3 hybrids), harvested at late-milk stage of maturity, and ensiled into mini-silos (3 silos×3 5 hybrids) for 60 d. After ensiling, silages were analyzed for composition and fermentation profile. 6 7 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 8 ANOVA, considering farm (F), hybrid (H), and  $F \times H$  interaction as variation sources. Incubation 9 10 (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 11 of silages were higher than those of the non-irrigated one (P<0.001) and the fermentation profile 12 13 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 14 15 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 16 significantly (P<0.01) influenced by hybrid (167, 200, 215 ml/g DM and 229, 257, 267 ml/g DM 17 for forage, sweet and grain genotypes after 24 and 48 h of incubation, resp.). The  $F \times H$  interaction 18 was significant for all considered parameters excluding DM, lignin, ash, pH, and in vitro 19 parameters. On the two farms, in general, forage and grain genotypes were largely different, 20 whereas the sweet sorghum was quite similar to the forage in one case or grain in the other. Results 21 22 of this experiment highlight the large variability of the nutritional values of sorghum hybrids grown in different conditions. 23

24

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

#### 27 Introduction

Silages obtained from sorghums belonging to conventional forage and grain genotypes were found 28 to be valid feed sources for dairy cows (Dann et al., 2008; Colombini et al., 2012). In the last years, 29 the potential of sorghum silage as ruminant feed has been evaluated also in Europe. Results would 30 suggest that the inclusion of such feed ingredient in dairy cow diets should be carefully considered, 31 as partial replacement, i.e., to corn silage (Colombini et al., 2010, 2012; Śliwiński et al., 2012). 32 33 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 34 specific chemical profile, some seed companies have been promoting sweet sorghum as a possible 35 36 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 37 fast and cost-effective analysis (Rymer et al., 2005). To date, only the study of Di Marco et al. 38 39 (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 40 comparing in vitro degradability, GP, and energy value of silages obtained from forage, sweet, and 41 grain sorghum grown in two farms located in the Po Valley (Northern Italy). 42

#### 43 Material and methods

44 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 45 (Surgo) and a grain sorghum (Favorite), both traded by SIVAM Spa (Casalpusterlengo, Lodi, Italy). 46 Plants were grown in two pilot farms of the Veneto Agricoltura Agency, one (Farm 1) located in the 47 province of Venice (Vallevecchia, latitude 45.6°N, longitude 12.9°E; 0 m above sea level) and one 48 (Farm 2) located in the province of Rovigo (Ceregnano, latitude 45.0°N, longitude 11.9°E; 5 m 49 above sea level). The farms were involved in a project aiming to evaluate quality of silages obtained 50 from different genotypes of sorghum. In each farm, sorghums were sown in nine experimental plots 51 (three plots per each hybrid) with an area of 0.2 ha each. Sowing took place in the first ten days of 52

June for all genotypes. No fertilizers were applied; urea (100 kg/ha) and herbicides were distributed 53 at post-emergence phase. Irrigation of plants occurred only in Farm 2 (on July 2), as the Farm 1 is 54 not equipped with an irrigation system. Sorghums were harvested on September 18, 2013 in Farm 1 55 and on September 12, 2013 in Farm 2, in order to collect from both sites plants at a late-milk stage 56 of maturity. The chemical composition of fresh forages was the following (expressed as mean value 57 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 58 forage sorghum (Bulldozer); DM, 5.8% CP, 58.5% NDF, 9.2% starch, 6.2% ash, for the sweet 59 sorghum (Surgo); % DM, 8.1% CP, 55.5% NDF, 21.0% starch, 6.6% ash, for the grain sorghum 60 (Favorite). After harvest, three aliquots of chopped forage (10 kg each) were prepared for each 61 hybrid, as a representative sample of the three experimental plots, homogeneously mixed, and 62 mechanically compacted into nine laboratory mini-silos (3 silos×3 hybrids) with 20 l capacity, 63 using a press equipped with a manometer and a hydraulic cylinder generating a compressive force 64 of 1.2 atm/cm<sup>2</sup>. The mini-silos were hermetically closed and stored for 60 d at  $24 \pm 3^{\circ}$ C. On 65 66 opening the mini-silos, the upper layer (10-15 cm) of silage was discarded, to limit risk of taking 67 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 68 followed on both farms. The first aliquot of each silage was sent to the laboratories of ARAV 69 70 (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 71 according to AOAC (2012). The NDF fraction, inclusive of insoluble ash, was measured with 72 Ankom<sup>220</sup> Fibre Analyzer (Ankom Technology, NY, USA). Ammonia N content and pH were 73 74 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 75 76 System AS3000 auto-sampler (Thermo Electron Corporation, Waltham, MA, USA), equipped with 77 an H<sub>2</sub>SO<sub>4</sub> 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 78

laboratories, samples were dried in a forced-air oven at 60°C for 48 h, to determine DM content, 79 and ground to 1-mm. Eight subsamples were prepared for each hybrid × farm combination and used 80 for in vitro tests. Fermentations were conducted with Ankom<sup>RF</sup> gas production (GP) system 81 (Ankom Technology, NY, USA). This system is a kit of bottles (310 ml) equipped with a pressure 82 detector and wireless connection to a PC. Each bottle was filled with feed sample (0.500±0.0010 g), 83 25 ml of rumen fluid, and 50 ml of buffer solution (ratio 1:2). Bottles were incubated at  $39 \pm 0.4$  °C 84 for 48 h and vented at 3.4 kPa, to avoid overpressure conditions (Cattani et al., 2014). Two 85 incubations were repeated in 2 successive weeks, and the following experimental design was 86 applied: 3 hybrids×2 farms×4 replicates, plus 4 blanks (bottles without feed sample), giving a total 87 88 of 28 bottles incubated in each of the two incubations. At the end of each incubation run, fermentation fluids were filtered into weighed crucibles (Robu Glasfilter-Geräte GMBH, Hattert, 89 Germany) and treated with a heat stable amylase, but without sodium sulphite, to assay residual 90 91 NDF, using a Fibertech Analyzer (VELP Scientifica, Milan, Italy). Rumen fluid was collected by an esophageal probe, as detailed by Tagliapietra et al. (2012), from three intact dry Holstein-Friesian 92 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 calculated as follows: 95

96 NDFd (% NDF) = [(NDF<sub>feed</sub> - NDF<sub>res</sub>)/NDF<sub>feed</sub>]  $\times$  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
- measured at 48 h (NRC, 2001; ME<sub>NRC</sub>) or GP measured at 24 h of incubation (Menke and
- 103 Steingass, 1988; ME<sub>Menke</sub>). The two equations were the following:

- 104 ME<sub>NRC</sub> (MJ/kg DM) =  $-0.45 \times 4.184 + 1.01 \times DE$
- 105 where DE is the digestible energy:
- 106 DE (MJ/kg DM) = [(NDFd/1000)  $\times$ 4.2 + (tdNFC/1000)  $\times$ 4.2 + (tdCP/1000)  $\times$ 5.6 + (tdFA/1000)
- 107  $\times 9.5 0.3$ ]  $\times 4.184$
- where NDFd is the NDF degradability (g/kg NDF) measured at 48 h; tdNFC, tdCP and tdFA are the
  estimated true digestible contents of non-fibre carbohydrates, CP and EE (g/kg DM) calculated
  using the equations proposed by NRC (2001) (i.e., Eqs. 2–4a to 2–4e).

111 ME<sub>Menke</sub> (MJ/kg DM) =  $2.20 + 0.1357 \times \text{GP24}_{200} + 0.0057 \times \text{CP} + 0.0002859 \times \text{EE}^2$ 

- 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*

Data of silage composition (proximate analysis, pH, fermentation acid profile, ammonia N) were 115 subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS 116 (SAS Institute Inc., Cary, NC, USA release 9.1). The statistical model considered effects of farm (2 117 levels: Farm 1 and Farm 2), hybrid (3 levels: Bulldozer, Surgo, and Favorite), and interaction 118 119 between farm and hybrid ( $F \times H$ ) as sources of variation. Other data (*in vitro* degradability, GP, and energy content of silages) were analyzed using a model that considered effects of farm, hybrid,  $F \times$ 120 H interaction, and, in addition, incubation run (2 levels: incubation 1 and incubation 2) as sources of 121 122 variation.

#### 123 **Results**

The DM content of silages was on average greater in Farm 2 compared to Farm 1 (29.0 vs. 25.5%, respectively; P<0.001; Table 1). The proximate composition of silages reflected the plant genotype. The forage sorghum had the greatest NDF, ADF, and ADL contents (P<0.001). On the other hand, the grain genotype showed the lowest fiber fraction, especially in Farm 1, and the highest starch content (P<0.001). As regards starch, the sweet genotype showed, on average, the lowest content in

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

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

#### 157 **Discussion**

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

ensiling into the mini-silos. Absence of significant effects due to the incubation run proves that the 181 182 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 183 vitro by Di Marco et al. (2009). In line with previous findings (Pesce et al., 2000; Bolsen et al., 184 2003), the grain genotype showed the greatest values of TDMd and GP, as a result of greater starch 185 content, whereas the forage sorghum showed the lowest values, as the fibrous fraction probably had 186 187 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 188 the other two hybrids. However, the sweet sorghum seemed to be closer to the forage genotype in 189 190 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 191 fermentation properties and energy value. 192

193 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 194 195 cultivated under different conditions. After ensiling, the sweet sorghum exhibited chemical 196 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 197 198 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 199 validated in vivo. 200

201

#### 202 Acknowledgements

This activity is part of Veneto Agricoltura Agency's project "Alternative crops rotation to contain diffusion of *Diabrotica virgifera virgifera* LeConte". The authors are grateful to the plant breeding companies, KWS Italia Spa and SIVAM Spa, for their kind support to the research, and to S. De Paoli (ARAV, Breeders Association of Veneto Region) for chemical analyses.

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	DM	Ether	CP	NDF	ADF	ADL	Ash	Starch
		extract						
Farm 1								
Forage	$22.3^{C}$	$2.0^{BC}$	$8.2^{BC}$	70.1 <sup>A</sup>	41.9 <sup>A</sup>	$4.6^{AB}$	$6.9^{AB}$	$5.8^{\mathrm{C}}$
Sweet	$22.8^{\circ}$	2.1 <sup>BC</sup>	$9.4^{AB}$	62.1 <sup>B</sup>	34.7 <sup>B</sup>	3.7 <sup>BC</sup>	7.6 <sup>A</sup>	4.4 <sup>C</sup>
Grain	31.3 <sup>A</sup>	3.3 <sup>A</sup>	$10.2^{A}$	49.3 <sup>D</sup>	27.4 <sup>D</sup>	$2.9^{\circ}$	$7.5^{\mathrm{A}}$	15.8 <sup>B</sup>
Farm 2								
Forage	26.7 <sup>B</sup>	2.1 <sup>BC</sup>	$8.2^{BC}$	72.0 <sup>A</sup>	42.3 <sup>A</sup>	5.2 <sup>A</sup>	$5.6^{\rm C}$	$4.6^{\mathrm{C}}$
Sweet	26.6 <sup>B</sup>	$2.4^{\text{B}}$	$8.7^{BC}$	$57.2^{BC}$	32.8 <sup>BC</sup>	$4.4^{AB}$	$6.4^{BC}$	13.2 <sup>B</sup>
Grain	33.6 <sup>A</sup>	1.6 <sup>C</sup>	$7.8^{\mathrm{C}}$	54.9 <sup>C</sup>	30.3 <sup>CD</sup>	3.9 <sup>AB</sup>	$6.5^{BC}$	20.0 <sup>A</sup>
$SEM^1$	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	***

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

Contrast significance is indicated ns=non-significant; \*P $\leq$ 0.05; \*\*P<0.01; \*\*\*P<0.001 A, B, C, D – values in columns with different letters differ significantly (P $\leq$ 0.01). <sup>1</sup>SEM = standard error of the mean 255

256

• 0 /	pН	Total FA	Acetate	Lactate	N-NH <sub>3</sub> /N
	PII	TotalTA	Actat	Lactate	11-1113/11
Farm 1		_			_
Forage	3.97 <sup>A</sup>	$14.4^{B}$	19.3 <sup>AB</sup>	$80.7^{\text{DE}}$	3.97 <sup>B</sup>
Sweet	3.89 <sup>AB</sup>	15.6 <sup>B</sup>	$20.4^{A}$	79.5 <sup>E</sup>	5.35 <sup>A</sup>
Grain	3.95 <sup>A</sup>	$18.2^{A}$	17.5 <sup>BC</sup>	82.4 <sup>CD</sup>	$6.54^{\text{A}}$
Farm 2					
Forage	3.74 <sup>CD</sup>	$14.8^{B}$	13.5 <sup>D</sup>	86.3 <sup>A</sup>	3.46 <sup>B</sup>
Sweet	3.62 <sup>D</sup>	17.9 <sup>A</sup>	14.1 <sup>D</sup>	85.6 <sup>AB</sup>	$2.97^{\mathrm{B}}$
Grain	3.81 <sup>BC</sup>	17.3 <sup>A</sup>	15.6 <sup>CD</sup>	$84.0^{BC}$	3.64 <sup>B</sup>
<sup>1</sup> SEM	0.032	0.34	0.56	0.56	0.317
Farm (F)	***	Ns	***	***	***
Hybrid (H)	***	***	ns	ns	***
F×H	ns	***	**	***	**

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-NH<sub>3</sub>/N; expressed as percentage) of three sorghum silages harvested in the two farms

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

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

 $^{1}$ 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

268 NRC (2001;  $ME_{NRC}$ ) or to Menke and Steingass (1988;  $ME_{Menke}$ ), of three sorghum silages 269 harvested in the two farms

	NDFd	TDMd	Gas production		Energy value		
	11214		24 h	48 h	ME <sub>NRC</sub>	ME <sub>Menke</sub>	
Farm 1							
Forage	57.3 <sup>a</sup>	69.6 <sup>bc</sup>	156 <sup>B</sup>	220 <sup>B</sup>	9.1 <sup>ab</sup>	$7.0^{\circ}$	
Sweet	54.5 <sup>abc</sup>	71.9 <sup>abc</sup>	181 <sup>B</sup>	236 <sup>B</sup>	$8.9^{b}$	$7.8^{\mathrm{BC}}$	
Grain	$50.2^{\circ}$	75.5 <sup>a</sup>	214 <sup>A</sup>	261 <sup>A</sup>	$9.7^{ab}$	8.9 <sup>A</sup>	
Farm 2							
Forage	$52.0^{bc}$	68.5 <sup>c</sup>	177 <sup>B</sup>	237 <sup>B</sup>	$8.9^{b}$	$8.0^{AB}$	
Sweet	51.7 <sup>bc</sup>	72.3 <sup>ab</sup>	219 <sup>A</sup>	278 <sup>A</sup>	$9.6^{ab}$	8.8 <sup>A</sup>	
Grain	55.7 <sup>ab</sup>	75.9 <sup>a</sup>	216 <sup>A</sup>	273 <sup>A</sup>	10.1 <sup>a</sup>	$8.6^{AB}$	
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	

270 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 ( $P \le 0.05$ ).

A, B, C – as above for  $P \leq 0.01$ .

 $^{1}$ SEM = standard error of the mean