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CHEMICAL COMPOSITION AND *IN VITRO* FERMENTATION OF SILAGES FROM DIFFERENT SORGHUM HYBRIDS CULTIVATED IN THREE PILOT FARMS

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Preliminary communication

SUMMARY

This experiment compared silages obtained from ten hybrids of sorghum grown in three farms of the Po Valley, in terms of in vitro degradability, gas production (GP), and energy value. Hybrids were sown on 30 experimental plots (three plots per each hybrid), harvested at late-milk stage of maturity and ensiled for 60 d into 30 mini-silos (3 silos×10 hybrids). After ensiling, silages were analyzed for proximate composition, pH, ammonia N, and fermentation acid profile. Degradability of NDF (NDFd) and of true dry matter (TDMd) was determined after 48 h of incubation using sequentially Daisy^{II} incubator and Ankom^{220} Fibre Analyzer. Two incubation runs (at 48 h) were carried out to evaluate in vitro GP of silages, and to estimate their energy content. All data were submitted to ANOVA considering "hybrid" and "farm" as sources of variation. The interaction between hybrid and farm was never significant and it was excluded from the statistical model. The contents of dry matter and NDF of silages were influenced by hybrid and farm ($P<0.001$). In contrast, the percentage of non-structural carbohydrates of silages was affected by hybrid ($P<0.001$) but not by farm. All chemical parameters were significantly affected by hybrid ($P<0.01$) and, except NCS, by farm ($P<0.05$). In vitro parameters (NDFd after 48 h and GP at 24 and 48 h of incubation) were influenced by hybrid and farm ($P<0.001$ and $P<0.01$), respectively. Among hybrids and farms, large differences ($P<0.001$) were also found out as regard to net energy content of sorghum silages. Because of this large variability, sorghum silages can be included successfully in ruminant diets considering the peculiarities of each hybrid with respect to the energy requirements of dairy cows.

Key-words: Sorghum hybrids, Sorghum silage, in vitro degradability, in vitro gas production, dairy cows

INTRODUCTION

Corn silage is the main ingredient of diets fed to dairy cows in farms of the Po plain (Italy), with the exception of those producing milk processed into some Protected Denomination of Origin cheeses. In the last years, corn production in the Po Plain has been complicated by the spread of mycotoxin contamination that reduces quantity and quality of corn silage and precludes its utilization for lactating cows. These concerns have given rise to the interest for alternative forages, which might partially or totally replace corn silage in diets. In this regard, sorghum (*Sorghum vulgare*) would represent an interesting crop, as plants adapt to different soils and are productive in conditions of water deficit (Sanchez et al., 2002). However, data about nutritional and energy value of sorghum hybrids commercially available are scarce. This experiment was aimed at comparing silages obtained from different sorghum hybrids grown in three farms of the Po plain, in terms of chemical composition, *in vitro* degradability, gas production (GP), and energy value.

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MATERIAL AND METHODS

Ten commercial hybrids of *Sorghum vulgare* spp., traded by four Italian seed companies, were analyzed (see Table 1). Hybrids were grown in three pilot farms of the Veneto Agricoltura Agency, Farm 1 located in the province of Treviso (Mogliano Veneto, latitude 45.3°N, longitude 12.1°E; 8 m a.s.l.), Farm 2 in the province of Rovigo (Ceregnano, latitude 45.0°N, longitude 11.9°E; 5 m a.s.l.), and Farm 3 in the province of Venice (Vallevecchia, latitude 45.6°N, longitude 12.9°E; 0 m a.s.l.). In all farms, sorghums were sown in 30 adjacent plots (three plots per each hybrid), with an area of about 28 m^2 each, at the first decade of June 2014. No fertilizers were applied; urea (100 kg/ha) was distributed at post-emergence phase in Farm 1, at pre-emergence phase in Farm 2 and 3. Herbicides were also distributed at post-emergence phase in Farm 2 and 3. Sorghums were harvested at the third decade of October 2014 in Farm 1, at the first decade of October 2014 in Farm 2 and 3, in order to collect plants being at late-milk stage of maturity. After harvest, three aliquots of chopped forage (10 kg each) were prepared for each hybrid, as representative sample of three experimental plots, homogeneously mixed, and ensiled into 30 laboratory mini-silos (3 silos per each hybrid) with a capacity of 20 l. The mini-silos were hermetically closed and stored for 60 d at 24±3°C. On the mini-silos opening, the upper layer (10-15 cm) of silage was discarded, to limit risk of anomalous fermentation. After that, two aliquots (about 1.5 kg each) were prepared for each sorghum silage, as representative sample of the three mini-silos. The same procedures were followed in all farms. The first aliquot was used to determine the proximate composition, pH, ammonia N ($N\text{-}NH_3$) content, and fermentation acid profile of silages. The analysis of proximate composition was conducted according to AOAC (2012). Values of pH were determined by a potentiometer equipped with a specific electrode (pH meter BASIC 20, Crison Instruments, Alella, Spain). The content of $N\text{-}NH_3$ was determined using a colorimetric method (Cataldo et al., 1975). Fermentation acids were measured using a Thermo Finnigan Spectra System AS3000 auto-sampler (Thermo Electron Corporation, Waltham, MA, USA), equipped with a H_2SO_4 0.0025 N Bio-Rad HPX-87H column (Bio-Rad Laboratories, Richmond, CA, USA). The second aliquot was used for *in vitro* tests. A pooled sample (about 500 g as fed) was dried in a forced-air oven at 60°C for 48 h for each silage to determine dry matter (DM) content, and then ground to 1 mm. Degradability of NDF (NDFd) and of true DM (TDMd) was determined after 48 h of incubation using sequentially Daisy^{II} incubator and Ankom^{220} Fibre Analyzer (Ankom Technology, NY, USA). Each filter bag was filled with 0.250±0.0010 g of feed sample (Cattani et al., 2009) and included in glass jars filled with rumen fluid and buffer solution (ratio 1:2). Rumen fluid was collected by an esophageal probe (Tagliapietra et al., 2012) from three intact dry Holstein-Friesian cows fed hay *ad libitum* and 2.5 kg/d of concentrates. Buffer solution was prepared according to Menke and Steingass (1988). The following experiment design was used: 1 run×10 hybrids×3 farms×2 replications, plus 8 blanks (bags without feed sample; 2 per each glass jar). Gas production was measured using Ankom^{RF} gas production (GP) system (Ankom Technology, NY, USA). This system is a kit of bottles (310 ml) equipped with a pressure detector and wireless connected to a PC. Each bottle was filled with feed sample (0.500±0.0010 g), 25 ml of rumen fluid, and 50 ml of buffer solution (ratio 1:2), collected as previously described. After filling, bottles were incubated at 39±0.4°C for 48 h and vented at 3.4 kPa, to avoid overpressure conditions (Cattani et al., 2014). Due to of the limited number of bottles, each silage was analyzed in two replicates, separately incubated in two successive runs. The resulting experimental design was: 2 runs×10 hybrids×3 farms, plus 10 blanks (bottles without feed sample; 5 per each run), giving a total of 70 bottles incubated (35 per each run). Metabolizable energy content (ME; MJ/kg DM) and milk forage unit (MFU; n°/kg DM) of silages were estimated according to Menke and Steingass (1988). Data of silage composition and of the various *in vitro* parameters were analyzed by the PROC GLM of SAS (SAS Institute Inc., Cary, NC, USA release 9.1), considering effect of hybrid and farm as sources of variation. The interaction hybrid × farm was excluded from the statistical model as it was never significant.

RESULTS AND DISCUSSION

The DM content and chemical composition of silages were influenced by hybrid and farm (Table 1). Within hybrids, the DM content ranged from 24.2 to 33.3%, respectively ($P<0.001$). The NDF content showed a great variability, ranging from 57.0 to 74.0% on DM ($P<0.001$). In turns, also the percentage of NSC (non-structural carbohydrates) was highly variable, being included between 14.9 to 27.1% on DM ($P<0.001$). Silages obtained in Farm 2 showed, on the average, the greatest DM ($P<0.01$) and NDF contents ($P<0.001$), whereas the mean content of NSC did not differ on the three farms. Chemical composition of silages was included in the expected ranges (NRC, 2001), even if the NDF content resulted, on the average, greater compared to the literature (Colombo et al., 2007; Colombini et al., 2010). Hybrids did not differ for final pH, which was always in the range (3.48-4.50) indicating proper fermentations during ensiling (Gallardo and Gagiotti, 2004). In contrast, final pH of silages resulted different on the three farms ($P<0.05$). The occurrence of correct fermentations in all hybrids was confirmed by high production of lactate, being the prevalent fatty acid (FA; on the average 77.1% on the total FA), followed by acetate (on the average 21.3% on the total FA); propionate was present only in traces, whereas n-butyrate was never detectable by HPLC (not shown by data). The ratio between $N\text{-}NH_3$ and total N was influenced by hybrid and farm ($P<0.01$) but it was always lower than the threshold of 7, which indicates a proper preservation of silages (Romero, 2004).

Table 1. Dry matter (DM, %), chemical composition (% on DM), pH, proportion of ammonia N on total N ($N\text{-}NH_3/N$), lactate and acetate (% on total fatty acids) of different sorghum hybrids

	DM	CP	NDF	ADF	ADL	Ash	NSC	pH	$N\text{-}NH_3/N$	Lactate	Acetate
Hybrid											
Argensil	24.9	5.0	58.1	35.4	4.4	9.1	25.8	3.82	2.48	78.6	20.5
Argensor	25.1	5.0	60.4	37.0	5.6	7.7	24.5	3.82	2.27	79.5	19.4
Biomass Mix	26.6	4.4	59.2	36.9	4.8	8.1	26.5	3.87	2.66	75.7	22.5
Buffalo Grain	24.2	5.6	62.2	36.1	4.0	8.1	22.1	3.84	3.30	78.5	20.5
Bulldozer	27.8	3.2	74.0	47.6	6.6	6.3	14.9	3.87	2.84	70.4	25.2
Freya	33.3	4.0	66.2	42.2	7.0	7.1	20.5	3.97	2.26	76.3	21.8
Hannibal	28.3	3.8	66.8	41.7	5.9	6.8	20.9	3.91	2.48	77.3	21.4
Little Giant	25.0	6.7	57.0	33.8	4.1	9.9	24.0	3.86	2.70	82.5	16.3
Mix Asolo Tris	25.1	5.1	58.4	35.2	4.4	7.3	27.1	3.79	3.64	76.0	23.3
Nectar	25.3	4.2	61.9	41.8	5.3	7.6	24.5	3.75	2.63	76.6	22.3
Farm											
Farm 1	27.0	4.7	62.7	40.5	0.7	7.1	23.4	3.80	2.12	77.1	21.7
Farm 2	27.5	3.4	65.3	38.9	0.3	7.7	21.9	3.83	2.19	74.8	23.4
Farm 3	25.1	6.0	59.3	36.9	0.7	8.5	24.1	3.92	3.70	79.6	18.8
P value											
Hybrid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	ns	<0.01	ns	ns
Farm	<0.01	<0.001	<0.001	<0.05	<0.001	<0.01	ns	<0.05	<0.01	<0.05	<0.01

CP=crude protein; NDF=neutral detergent fibre; ADF=acid detergent fibre; ADL=acid detergent lignin; NSC=non-structural carbohydrate

Values of *in vitro* NDFd were influenced by hybrid and farm ($P<0.001$) showing a great variability, ranging among hybrids from 39.6 to 61.8% on NDF, and among farms from 47.9 to 55.3% on NDF (Table 2). A large variability of *in vitro* NDFd among different hybrids has been also reported by Di Marco et al. (2009). As the main result of large differences in chemical composition, the ten hybrids produced different amounts of gas, both at 24 (GP24) and at 48 h (GP48) of incubation ($P<0.01$). Likewise, hybrids differed largely ($P<0.01$) for energy content. In terms of ME, hybrids ranged from 7.4 to 9.5 MJ/kg DM; in terms of MFU silages ranged from 0.59 to 0.80 MFU/kg DM. These values agree with data reported by main feeding systems for ruminants (INRA, 1988; NRC, 2001). No differences among farms were found out for GP24 and, consequently, for energy content (ME and MFU) of sorghum silages ($P>0.05$). However, large differences were observed as regard to biomass yield provided by the different hybrids, varying from 9.7 to 17.3 t DM/ha ($P<0.001$), and obtained on the three farms varying from 9.6 to 14.4 t DM/ha ($P<0.001$). However, such yields are in line with ranges declared by seed companies. As consequence, also the MFU produced per hectare showed a great variability, ranging from 6731 to 11250 MFU/ha among hybrids ($P=0.001$), and from 6563 to 10040 MFU/ha among farms ($P<0.001$).

Table 2. *In vitro* degradability of NDF (NDFd, % on NDF) and of true dry matter (TDMd, % on DM), gas production at 24 h (GP24; ml/g DM) and 48 h of incubation (GP48; ml/g DM); metabolizable energy (ME; MJ/kg DM) and milk forage unit (MFU[1]; expressed as n°/kg DM); biomass yield (BY; t DM/ha) and milk forage unit produced per hectare (MFU[2]; expressed as n°/ha) of different sorghum hybrids

	NDFd	TDMd	GP24	GP48	ME	MFU[1]	BY	MFU[2]
Hybrid								
Argensil	51.9	74.3	231	289	8.9	0.74	10.6	7829
Argensor	48.7	71.4	213	276	8.4	0.69	11.5	7961
Biomass Mix	49.8	72.3	224	283	8.6	0.71	12.4	8772
Buffalo Grain	61.8	78.3	253	312	9.5	0.80	10.9	8647
Bulldozer	48.9	64.6	191	269	7.7	0.62	14.7	8882
Freya	39.6	62.8	179	244	7.4	0.59	11.5	6731
Hannibal	48.3	67.8	203	266	8.0	0.65	17.3	11250
Little Giant	53.4	75.4	219	271	8.7	0.72	9.7	7028
Mix Asolo Tris	55.6	76.1	233	295	9.1	0.76	12.6	9507
Nectar	49.1	70.9	222	286	8.6	0.70	15.8	11166
Farm								
Farm 1	48.6	69.7	222	280	8.7	0.68	14.1	10040
Farm 2	55.3	72.7	216	289	8.3	0.68	9.6	6563
Farm 3	47.9	71.4	212	268	8.4	0.69	14.4	9729
P value								
Hybrid	<0.001	<0.001	<0.01	<0.01	<0.01	<0.01	<0.001	0.001
Farm	<0.001	<0.05	ns	<0.05	ns	ns	<0.001	<0.001

CONCLUSION

The results of the study provide evidence that sorghum silages can largely differ for chemical composition, *in vitro* parameters, energy value, and biomass yield. Thus, cultivation and inclusion of this forage in dairy cow diets must inevitably consider peculiarities of each hybrid in relation to the energy requirements of animals (sorghum silages with lower energy content could be successfully used for animals with low energy requirements; i.e. heifers or dry cows). However, preliminary results presented in this paper should be validated by *in vivo* experiments.

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