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Mixed hay produced in Southern Italy: nutritive value and environmental impact

Alessandro Vastolo[1], Dieu donné Kiatti[1], Monica Isabella Cutrignelli[1], Serena Calabrò[1]

[1] *Department of Veterinary Medicine and Animal Production DMVPA, University of Napoli Federico II Napoli, Italy*

ABSTRACT
The aim of this study was to characterize eight mixed forages preserved as hays produced in four agricultural and livestock farms located in four Provinces (Avellino, Benevento, Campobasso, and Potenza) of Southern Italy, different for environmental condition. Nutritive value, *in vitro* fermentation characteristics and kinetics, including methane production were determined. The little differences observed between sampling areas highlighted that the forage produced in Avellino area is the most interesting in terms of chemical composition, nutritive value, *in vitro* characteristics, and environmental impact. Data obtained allow having more information about hays produced in the study area, useful for farmers to make balanced rations, to maintain animal health and guarantee high quality of production.

Section: RESEARCH PAPER

Keywords: mediterranean area; forage; in vitro fermentation kinetics; methane production; volatile fatty acids

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Corresponding author: Alessandro Vastolo, e-mail: alessandro.vastolo@unina.it

1. INTRODUCTION

Administration of fresh forages for livestock is not everywhere possible during the whole year because their production is limited only to the most favourable seasons. Therefore, it is necessary to store forages to take advantage of stock throughout the year. Haymaking involves the transformation of grass into hay thanks to a progressive loss of moisture that reaches 15-20 % [1]. Haymaking is one of the most popular and traditional systems of forage conservation in Italy. In many regions of Mediterranean area (Southern Italy), the forage preserved as hay is produced in the hilly and mountain area of Appennino (Province of Avellino, Benevento, Potenza, Campobasso). This hay is utilized in ruminant farms, either extensive to produce principally high-quality meat, milk, and dairy products or intensive to produce mainly Mozzarella di Bufala Campana DOP. As known, forages present extremely variable chemical composition depending on many factors [2]. A high influence is given by the environmental condition in which forages are grown, including geographical variation, climatic conditions (i.e., rainfall, temperature, humidity), soil type (i.e., sand, silt, and clay) and characteristics (i.e., pH, water-holding capacity, fertility) and the stage at harvesting [3]. Knowledge of hay nutritive value is important for balancing rations to maintain animal health and guarantee high level of production, in terms of quality and quantity. In addition, considering the increasing attention of public opinion and researchers for the environmental impact of livestock, when forage represent a high incidence in ruminant diets, is also very important consider its greenhouse gas (GHG) emission. The use of locally hay has economic advantages for the breeder but also environmental rewards in terms of global warming potential as reported in a Life Cycle Assessment (LCA) study [4], [5].

The trial reported has been performed by *in vitro* cumulative gas production technique (IVGPT), a method ethically advantageous, faster, and less expensive than *in vivo* techniques. The benefits of the IVGPT also include the possibility to run large batches simultaneously and the ability to measure fermentation kinetics of a single feedstuff. The IVGPT is essentially based on anaerobic digestion of fermentable carbohydrates by microorganisms. On this basis, it is possible to

obtain information on fermentation kinetics, organic matter degradability, and fermentation end-products (e.g., volatile fatty acids, ammonia). In the last decade, the IVGPT was also suggested to measure the methane (CH_4) production. In this regard, IVGPT can represent a valid instrument to preliminary test nutritional strategies to reduce GHG emissions in animal production: for instance, evaluating feedstuff or bioactive molecules able to limit the action of methanogens bacteria. The application of IVGPT could be useful to determine the influence of CH_4 inhibitors on fermentation patterns and include CH_4 as a new criterion in diet formulation.

The objective of this investigation was to characterize forage preserved as hay produced in different agricultural and livestock farms of Mediterranean area, in terms of nutritive value, *in vitro* fermentation characteristics, including CH_4 production. The hypothesis is that different areas of sampling influence the hay characteristics due to environmental conditions.

2. MATERIAL AND METHODS

2.1 Experimental design

For the study, eight mixed hay samples produced in four different areas of Southern Italy: Avellino (AV), Benevento (BN), Campobasso (CB) and Potenza (PZ) provinces, were collected (Table 1). Two samples for each area in two different agricultural-livestock farms were collected. All samples were transported to the Feed Analyses Laboratory of the DMVPA (University of Napoli Federico II, Napoli, Italy), where they were ground to pass a 1 mm screen and analysed for chemical composition, *in vitro* fermentation kinetics and characteristics, including CH_4 production [6], [7].

2.2 Nutritive value

The hay samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to official protocol by Association of Official Analytical Chemists [8] procedures (ID number: 2001.12, 978.04, 920.93, 978.10 and 930.05 for DM, CP, EE, CF and ash, respectively). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were also determined [9].

The hays' nutritive value, expressed as net energy for lactation (Unité Fourragère du Lait, UFL), was calculated with two methods: i) as suggested by INRA [10] utilizing the chemical composition parameters (DM, CP, EE, CF and ash); ii) as suggested by Menke and Steingass [11] utilizing Equation (1):

$$NE = 0.54 + 0.0959 \cdot GP_{24} + 0.0038 \cdot CP + 0.0001733 \cdot CP^2, \quad (1)$$

where *CP* is expressed in g/kg DM and GP_{24} (gas produced after 24 h of fermentation, see below) as ml/200 mg DM.

The *in vitro* fermentation characteristics were studied incubating the substrates at 39 °C (in six replications) under anaerobic conditions with cow inoculum. In particular, the substrates were weighed (1.007 ± 0.0061) g in 120 ml serum bottles and 75 ml of anaerobic medium were added. The rumen fluid was collected in a pre-warmed thermos at a slaughterhouse authorized according to EU legislation (Regulation EC No. 882/2004) from four dairy cows (mean body weight 680 kg) fed a total mixed ratio containing corn silage, oat hay and concentrate (CP: 12.0 and NDF: 43.5 % DM). The collected material was rapidly transported to the laboratory, where it was pooled, flushed with CO_2, filtered through cheesecloth, and added to each bottle (10 ml). Gas production was recorded 24 times (at 2 to 24 h intervals) during the period of incubation using a manual pressure transducer. The cumulative volume of gas produced after 120 hours of incubation was related to the incubated organic matter (OMCV, ml/g). When the fermentation was stopped the fermenting liquor was analysed for pH and sampled for volatile fatty acids (VFA) determination. In particular, the fermenting liquor was centrifuged at 12,000 g for 10 min at 4 °C and 1 ml of supernatant was mixed with 1 ml of oxalic acid (0.06 mol). VFA were detected by gas chromatography equipped with a fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), using an external standard solution composed of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids. The extent of sample disappearance, expressed as organic matter degradability (dOM %), was determined by weight difference of the incubated OM and the undegraded filtered residue burned at 550 °C for 5 h. For each gas run, three bottles were incubated without substrate (blanks) to correct dOM, OMCV and VFA. After 24 hours of incubation CH_4 production was determined stopping 3 bottles/samples and collecting 3.0 ml of the head-space gas in duplicate with a gastight syringe. The analysis was carried out using a gas chromatograph equipped with a loop TC detector and a packed column. At 24 h dOM was also measured [12].

To estimate the fermentation kinetics, for each bottle, the gas production profiles were fitted to the following model [13]:

$$G = \frac{A}{1 + \left(\frac{B}{t}\right)^C}, \quad (2)$$

where *G* is the total gas produced (ml per g of incubated OM) at time *t* (in h), *A* is the asymptotic gas production (in ml/g), *B* is the time at which one-half of *A* is reached (in h), and *C* is the curve switch. Maximum fermentation rate $R_{\max}$ ml/h and the time at which it occurs $T_{\max}$ h were calculated utilizing model parameters [14]:

$$T_{\max} = C\left(\frac{B-1}{B+1}\right)^{1/B}, \quad (3)$$

$$R_{\max} = A\,B\,C^B\,\frac{T_{\max}^{B-1}}{1+C^B}\,(T_{\max}^{-B})^2. \quad (4)$$

Table 1. Geographical position, environmental condition of the hay sampling areas. The following abbreviations are used: *Long* - longitude; *Lat* - latitude; *Alt* - altitude. *T* represents the temperature.

Site	*Long* (°E)	*Lat* (°N)	*Alt* (m a.s.l.)	*T* (°C)	*Rainfall* (mm)
Formicoso (AV)	14.47	40.54	360	13.9°C	1354
Castelpagano (BN)	14.46	41.07	154	15.1°C	787
Campochiaro (CB)	14.40	41.33	701	12.3°C	583
Paterno (PZ)	15.48	40.38	760	11.3°C	650

2.3 Statistical analysis

The statistical significance of the differences was determined by ANOVA one way (SAS, 2000) according to the model:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}. \quad (5)$$

3. RESULTS AND DISCUSSION

The botanical essences more representative individuated in the mixed hays were *Onobrychis viciifolia* and *montana*, *Hedysarum*

Table 2. Nutritive value of the hays produced in the sampling areas. The following abbreviations are used: AV: Avellino; BN: Benevento; CB: Campobasso; PZ: Potenza *DM*: dry matter; *CP*: crude protein; *NDF*: neutral detergent fiber; *ADF*: acid detergent fiber; *ADL*: acid detergent lignin. *UFL** calculated as in [11]). *UFL†* estimated as proposed by [10]. *MSE*: Mean square error;
Different letters (a-c) within the column: differences statistically significant for $P < 0.05$. ***; $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, NS: not significant.

Area	*DM* (%)	*CP* (%)	*NDF* (%)	*ADF* (%)	*ADL* (%)	*Ash* (%)	*UFL** (/kg DM)	*UFL†* (/kg DM)
AV	89.84 b	14.19 a	42.13 b	29.83 b	5.17	11.99 a	0.65 a	0.66 a
BN	92.06 a	10.08 ab	54.05 a	38.69 a	5.81	9.25 b	0.41 ab	0.61 ab
CB	92.32 a	7.92 b	58.63 a	39.04 a	4.66	9.21 b	0.28 b	0.57 b
PZ	90.60 ab	12.59 ab	49.95 ab	35.12 a	5.01	8.60 b	0.56 ab	0.62 ab
Prob. t	**	*	**	***	NS	***	*	*
MSE	0.895	2.345	4.960	1.944	1.238	0.734	0.143	0.033

coronarium, *Trifolium pratense*, *subterraneum* and *brachycalycinum*, *Medicago sativa* and *polymorfa*, for Leguminosae and *Lolium perenne* and *rigidum*, *Dactylis glomerata*, *Festuca arundinacea* and *pratensis*, *Poa pratensis*, *Phleum pratense* and *alpinum* for Graminacee, the most common forage species produced in the areas of interest [11], [12].

All chemical composition parameters (Table 2) resulted statistically influenced by sampling area, excepted ADL. In general, the CP level ranged from 7.92 % to 14.20 % DM and NDF content between 42.13 % and 58.63 % DM, indicating a medium quality as forage to including in ruminant diets. On the other hand, the hay produced in Campobasso area showed the less advantageous values for the CP, NDF and UFL. The nutritional value (UFL/kg DM) estimated using INRA method is in every case higher than calculated using *in vitro* parameters. However, both systems ranked the area in the same way (AV > PZ > BN> CB). The major differences emerged in samples with lower net energy content (BN and CB), in which UFL obtained with Menke and Steingass [11] resulted very low (0.41 and 0.28, respectively). The gas produced after 24 h of incubation, quite low in these substrates, contributed to this result. As results, the nutritive value calculated with INRA system, only using chemical data, leads to an overestimation of energy availability, mainly in the forage of low quality, compared to a method that use a biological approach.

Comparing sampling area (Table 3), except for OMCV, statistically significant differences are observed for some parameters [dOM, T_{max} ($p < 0.001$) and R_{max} ($p < 0.05$)]. In particular, hays produced in PZ area were more degradable (dOM: 70.29 %, $p < 0.05$), characterized by a more rapid fermentative process (R_{max}: 9.64 ml/h and B: 17.5 h, $p < 0.05$). On the other hand, CB produced hays characterized by the lowest degradability (dOM: 60.77 %, $p < 0.05$) and slower fermentation kinetics (R_{max}: 7.20 ml/h and B: 20.9 h, $p < 0.05$). Regarding the *in vitro* fermentation kinetics (Figure 1) of analysed hays, the fermentation rate over time shows different shape for AV and BN: BN showed a faster fermentation process compared to AV (T_{max}: 6.20 vs. 8.88 h, $p < 0.05$; R_{max}: 8.60 vs. 7.64 ml/h, for BN and AV, respectively). Some clear differences appear for PZ, characterized by a more rapid and intense fermentation process and for CB with a slower and less intense gas production.

Concerning volatile fatty acids and pH measured after 120 h of incubation (data not showed), statistical differences ($p < 0.01$) appear in hay samples of the sampling areas only for iso-butyric, iso-valeric and valeric acids. These three branched-chain fatty acids formed in the rumen, generally in small quantities, by deamination of amino acids: iso-butyric acid from valine, valeric acid from proline, 2-methyl butyric acid from isoleucine and 3-methyl butyric acid from leucine [13]. The total VFA are higher in AV and CB and lower in BN and PZ, and the same tendency is present in acetic and propionic acid. The pH values after 120 h of incubation are adequate for the fermentation of cellulolytic bacteria [15], even if slightly low for CB (pH: 6.08).

The highest methane production was measured in hays from PZ area, associated to the high acetate (58.5 mmol/g) and butyrate (7.56 mmol/g) production. On the contrary, CB area hay produced less methane associated to the highest propionate acid production (19.9 mmol/g). McAllister *et al.* [16] and Moss *et al.* [17], argued that in the *in vitro* study the methane production is related to volatile fatty acids, acetate and butyrate promote the methane production while the propionate formation can be considered as a competitive pathway for hydrogen use in the rumen.

The lowest methane production was measured in CB (iCH_4: 9.18 ml/g) (Figure 2), which however, also showed the lowest dOM (40.9 %), probably due to the highest structural carbohydrates content (NDF: 58.63 % DM). On the contrary,

Table 3. *In vitro* fermentation characteristics at 120 h of hays produced in the sampling areas. AV: Avellino; BN: Benevento; CB: Campobasso; PZ: Potenza. *dOM*: organic matter degradability; *OMCV*: cumulative gas production related to incubated organic matter; T_{max}: time at which maximum rate was reached; R_{max}: maximum fermentation rate. *MSE*: Mean square error. Different letters (a-c) within the column: differences statistically significant for $P < 0.05$. ***; $P < 0.001$; **: $P < 0.01$, NS: not significant.

Area	*dOM* (ml/g)	*OMCV* (ml/g)	T_{max} (h)	R_{max} (ml/h)
AV	69.36a	252	8.88a	7.64ab
BN	60.77c	250	6.20b	8.60ab
CB	64.81b	230	8.32a	7.20b
PZ	70.29a	260	7.38ab	9.64a
Prob. t	***	NS	***	**
MSE	3.57	461	1.51	2.03

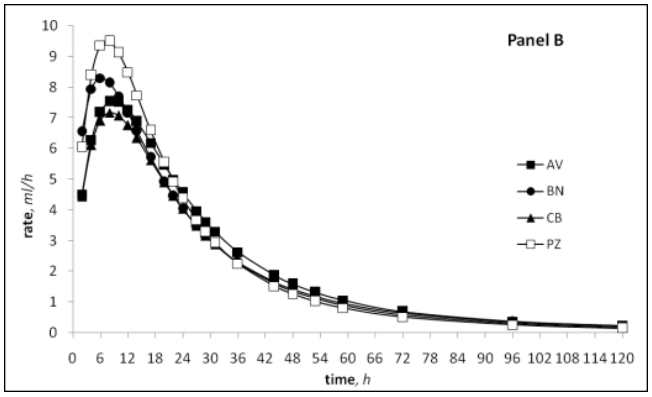

Figure 1. *In vitro* fermentation rate of hays produced in the sampling areas.

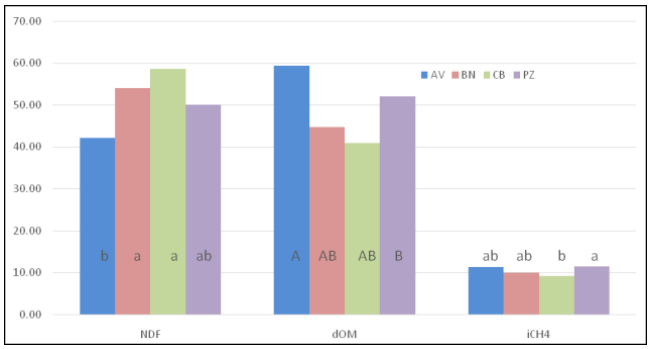

Figure 2. *In vitro* fermentation characteristics after 24 h of incubation of hays produced in the sampling areas. AV: Avellino; BN: Benevento; CB: Campobasso; PZ: Potenza. NDF: Neutral detergent fiber (% DM). dOM: organic matter degradability (%); iCH4: methane production related to incubated OM (ml/g). A-B and a-b: differences statistically significant for $p < 0.001$ and $p < 0.05$, respectively.

AV showed the highest methane production (iCH_4: 11.25 ml/g), OM degradability (59.3 %) and a low NDF content (42.13 % DM). These results are also in line with the nutritive value (UFL/kg DM: 0.65 vs. 0.28, for AV and CB respectively) and maybe influenced by CP content (14.19 vs. 7.92 % DM, for AV and CB respectively). Not so clear is the role of hemicellulose content, higher in CB and lower in AV (19.59 % and 12.30 % DM, respectively) and maybe influenced by CP content (14.19 % vs. 7.92 % DM, for AV and CB respectively).

Little differences among sampling areas were observed regarding all parameters considered (i.e., chemical composition, *in vitro* fermentation characteristics and methane production). However, the best area sampling, in terms of chemical composition (CP and NDF content) and nutritive value (UFL/kg DM) was the forage produced in Avellino area that also showed the most interesting *in vitro* characteristics (high OM degradability, gas and VFA production) and favourable environmental characteristics (low CH_4 production). On the other hand, the forage produced in Benevento area resulting medium in chemical composition, low in OM degradability and VFA production, and medium in CH_4 production; whereas Campobasso area produced forage with low environmental impact but characterized also by a less favourable nutritive value. These considerations are in both case not in according to the sensory evaluation (data not showed). The hay produced in Potenza province, showed better characteristics in terms of sensory evaluation and *in vitro* parameters, not completely in accordance with the chemical composition and environmental impact. Considering the environmental condition of the areas covered by the studies, we can say that probably the low altitude (360 m a.s.l.) of this area, but also the high rainfall and intermediate temperature recorded in the sampling year (1354 mm and 13.9 °C, respectively) in the Avellino area favoured the production of a best quality hay compared to the other areas. On the contrary, the environmental condition of Campobasso area, sited in at higher altitude (701 m a.s.l.) and characterized by low rainfall and intermediate temperature (583 mm and 12.3 °C, respectively), are in part responsible of the lower quality of hay. In the area of Potenza, was produced a good quality forage probably due to high altitude (760 m a.s.l.), not very rigid temperature (11.3 °C) and low rainfall (650 mm). The most important environmental factors that influence forage quality are temperature, water deficit, solar radiation, and soil nutrient availability, particularly, temperature usually has the greatest influence [17].

Optimal growth temperatures are near 20 °C for cool-season species such as alfalfa, orchardgrass, and ryegrass [18]. At temperatures below the optimum for growth, soluble sugars accumulate because of the lower temperature sensitivity of photosynthesis compared with that of growth. A rise in temperature normally increases rate of plant development and reduces leaf/stem ratios and digestibility. Increasing temperature lowers forage quality even when compared at the same morphological stage. The depressed digestibility associated with elevated temperatures is usually attributed to higher NDF concentrations [19]-[20]. Additionally, the NDF of forages grown under higher temperatures is usually less digestible than that of forages grown under lower temperatures because of increased lignification [21].

4. CONCLUSIONS

Studied forages presented a rather variable chemical composition that could depend on many factors (i.e., environmental condition of the sampling area during the plant growth and harvest, as well as the haymaking technique, but also the botanical species present in the hays), which may have influenced the *in vitro* fermentation characteristics, including methane production. Data obtained from this study provide some useful information about forages produced in some provinces of Southern Italy, where high quality dairy products are produced. The knowledge of these characteristics and the need to improve them, if necessary, is required by the farmers to make balanced rations to maintain animal health and guarantee high level of production, in terms of quality and quantity.

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