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An *in vitro* study of the total amount of cumulative gases produced of alfalfa, hay, straw, silages and grass masses from different cattle farms in Bulgaria

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Abstract: The amount of gas formed in different types of bulky coarse forages at 24h and 48h incubation is different and depends on the rate of dry matter decomposition. The mean values of the alfalfa samples from the three farms was 244.5 ± 43.6 ml/g DM at 24 h incubation and 288.4 ± 50.1 ml/g DM at 48 h incubation.
The mean values of the corn silages samples from the fourteen farms was 199.8 ±19.3 ml/g DM at 24 h incubation and 259.3±18.2 ml/g DM at 48 h incubation.
The mean values of the senages, grass masses samples from the eight farms was 115.1 ± 11.07 ml/g DM at 24 h incubation and 162.4 ± 15.6 ml/g DM at 48 h incubation.
The mean values of the straw samples from the twelve farms was 144.6 ± 56.2 ml/g DM at 24 h incubation and 205.1 ± 56.6 ml/g DM at 48 h incubation.
The mean values of the alfalfa hay samples from the five farms was 208.3 ± 28.3 ml/g DM at 24 h incubation and 239.9 ± 33.2 ml/g DM at 48 h incubation.
In the case of forages - straw, silage and haylage, the gas formed at 48 h incubation was 29.74% to 41.80% more compared to 24 h.
The amount of gas formed as a consequence of fermentation processes in ruminants studied by the "*in vitro*" gas production method depends mainly on the specific chemical composition and structure of the feed.

Keywords: *in vitro* gas production; enteric fermentation; bulky coarse forages; ruminants

INTRODUCTION

Several systems are available to measure gas production (GP) during *in vitro* rumen fermentation. Many of these systems are equipped with devices to release gas at fixed time intervals (Theodorou et al., 1994), or at fixed pressures (Cone et al., 1996; Davies et al., 2000; Calabrò et al., 2005; Tagliapietra et al., 2010). Gas venting is recommended to avoid pressure and conditions that cause partial dissolution of carbon dioxide (CO_2) in the fermentation liquid, resulting in underestimation of total gas production (Tagliapietra et al., 2010) and possible disruption of microbial activity (Theodorou et al., 1994), hence methane (CH_4) production. Closed systems are more commonly used to measure methane production. In closed systems the gas is not vented and remains in the bottle until it is collected for analysis (Pell and Schofield, 1993; Getachew et al., 2005; Pellikaan et al., 2011). However, the gas pressure generated at the top will cause partial dissolution of CO_2 in the air, which can change the composition of the gas output, so an adjustment for dissolved CO_2 is needed. The difference in the concentration of CO_2 in the headspace and the amount dissolved in the liquid can in turn change the concentration of methane (CH_4) in the collected gas (Patra and Yu, 2013).

In vitro gas production (IVGP) experiments, which involve the incubation of forages with rumen contents, are widely used to assess the nutritional value of forages for ruminants. Measurements based on the gas production technique (IVGP) complement standard laboratory analysis of chemical composition and therefore offer a rapid and less expensive alternative to "*in vivo*" nutrient digestibility studies (Rymer et al., 2005). Recently, IVGP techniques have been used to evaluate the potential of ration, nutritional (feed) ingredients and rumen fermentation modifiers to reduce methane (CH_4) emissions from ruminants (Bodas et al., 2008; Durmic et al., 2010). Chemical analyses provide good information on forage quality, but not enough information to determine the true nutritive value of the forage (Cherney, 2000). Since forage utilization is largely dependent on microbial degradation in the rumen, the description of forages in terms of their degradation is interesting. Menke et al., (1979) found high accuracy in predicting organic matter decay in "*in vivo*" studies using "*in vitro*" measurement of gases and chemical composition in a multiple regression equation. Groot et al., (1996) introduced a three-phase model into gas production (GP) kinetics that distinguished between soluble, insoluble but fermentable feed and microbial change. This model provides useful data on gas production (GP) kinetics and the model has proven to be an alternative to Tilley & Terry, (1963) and the plastic bag technique (Cone et al., 1998). Multiphase models are more flexible than single phase models and can give a better description of the degradation of different feed components (Groot et al., 1996). This model also allows to describe cumulative greenhouse gases (GP) very accurately during the first hours of gas production (GP), which is not possible in many other models (Cone et al., 1996). The gas production technique can be a powerful tool for research on the degradability of complex forages such as grasses, legumes and other crops. It is used to obtain additional information on nutrient utilization and the efficiency, with which the animal utilizes forage nutrients (Getachew et al., 1997). This method is not only useful for measuring the degradability of a given forage, it can also detect differences in fermentability between different chemical and physical pretreatments of straw, the fermentability of forage crops grown under different environmental conditions (Williams, 2000), and how different ensiling methods may affect forage consumption (Beuvink & Spoelstra, 1994). The most significant factors related to the "*in vitro"* gas production technique are the procedures used to collect and process the rumen fluid (Cornou et al., 2013), the composition of the buffer (Patra and Yu, 2013), the type of equipment (Gierus et al., 2008; Tagliapietra et al., 2010; Cattani et al., 2014), and the ratios between the feed and fermentation fluid sample sizes (Ramin and Huhtanen, 2012).

In vitro gas production is a widely used method of evaluating the energy value of several classes of the forages (Getachew et al., 1998), in particular straw (Makkar et al., 1999), agro-industrial by-products (Krishna and Günther, 1987), combined feed (Aiple et al., 1996) and various tropical feed (Krishnamoorthy et al., 1995).

The present study is designed to establish the total amount of gases produced in alfalfa, silages, grass masses, straw and alfalfa hay, fermented with rumen content by an "*in vitro*" system is important implications for elucidating the role of this group of staple forages in summer feeding of herbivores and raw material for the preparation of staple roughages for winter in ruminant rations.

The aim is to investigate the total amount of cumulative gases produced "*in vitro*" in alfalfa, straw, alfalfa hay, corn silages, senages and grass masses.

MATERIAL AND METHODS

Forage samples tested are alfalfa n = 3, silages n = 14, senages and grass masses n = 8, straw n = 12 and alfalfa hay n=5 from different regions of the country and analyzed for the content of structural fibrous components and*"in vitro"* fermentation. In the table, the mean values of the investigated forages with 3 replications are taken. Conditions of the study. Source of rumen fluid - three rams (Breed: SPBM - Synthetic Population

Bulgarian Milk); location: Agricultural Institute - Stara Zagora; taking rumen fluid from the stomach - in the morning before feeding. Incubation method: RF - two incubations: 24 h and 48 h.

Samples required amount of feed (0.5 g feed ± 0.001) per each module.The rumen content required for each module of feed is 25 ml. Medium solution is used - 50 ml for each feed module. The *in vitro* gas production technique is based on the relationship between rumen fermentation and the gases produced. The analysis is performed using the gas production technique *Ankom Gas Production System* ^{RF} *(Ankom, Tech. Co., Fairport, NY, USA*).

Gas production data are expressed as gas produced (ml) per gram of dry matter incubated (GP ml/g incubated dry matter DM) in 2 incubation periods 24 h and 48 h. Changes in gas pressure after 24 h and 48 h of fermentation are accumulated (ΔP) and converted to volume units using the ideal gas law:

GP (ml/g DM) = (ΔP/Po) x Vo,

where ΔP is the change in accumulated pressure (kPa) at the top of the module; Vo is the volume of the bottle at the top- (235 ml), Po is the atmospheric pressure read by the equipment before incubation starts. The bottles are placed in a ventilated incubator at 39 ± 0.5 ◦C for 48 h of incubation, avoiding that the pressure in the bottles exceed 48 kPa (Theodorou et al., 1994).

Statistical analyzes

All experimental data are statistically analysed by the software product SYSTAT 12 © *Copyright 2007, SYSTAT Software, Inc. All Rights Reserved and STATSOFT Statistics for Windows 10.* Mean values (*X̄*); standard deviation (*SD*); minimum (*Min*) and maximum (*Max*) values are determined by *Descriptive Statistics - brake down one way ANOVA*.

RESULTS AND DISCUSSION

Figure 1 shows the cumulative gases produced by alfalfa from different regions of the country.

The lowest values of cumulative gas production for 24 h of incubation are found in the alfalfa sample from farm № 2 with 176.6 ml/g DM, and the highest in the one from farm № 3 with 314.2 ml/g DM of gas produced.

Gas production for 48 h of incubation shows the lowest values for the alfalfa sample from farm № 2 at 250.6 ml/g DM, and the highest values are obtained from farm №3 at 349.0 ml/g DM. The variation decreased slightly for both samples.

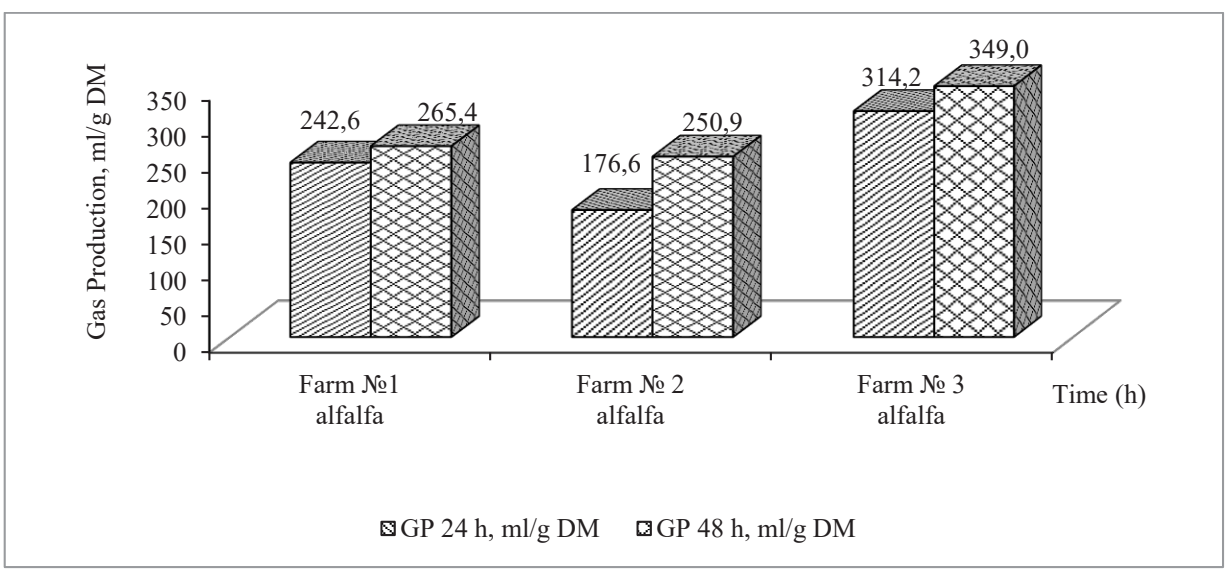

Figure 1. Analysis of the total amount of produced gases GP (ml/g DM) during 24h and 48h incubation of feed alfalfa from different farms in Bulgaria

The mean values of the alfalfa samples from the three farms was (GP, 24h) - 244.5 ± 43.6 ml/g DM at 24 h incubation and (GP, 48h) - 288.4 ± 50.1 ml/g DM at 48 h incubation.

Figure 2 Shows the gases produced by silages at both incubation times.

From the data in the figure, it can be seen that the gas production from maize silage varies across regions and farms. With the lowest values of gases produced at 24 h of incubation is corn silage at farm № 1 - 54.9 ml/g DM, and the highest of corn silage from farm № 6 - 377.8 ml/g DM of gas produced.

Similar values are found by Tagliapietra et al., (2011) in corn silages in an "*in vitro*" study at 24h incubation, the following values, respectively: (GP24) -273±20 ml/g DM; (GP24) - 245±25 ml/g DM and (GP24)- 275±20 ml/g DM. At 48 h of incubation, the highest values are the gases produced from the corn silage of farm № 6- 444.8 ml/g DM, and the lowest values are in the corn silage of farm № 1- 125.6 ml/g DM. The silages increase their cumulative gas production from 199.8 ml/g DM to 259.3 ml/g DM, maintaining their ranking. Sample variation is less compared to that of other forages.

The mean values of the corn silages samples from the fourteen farms was 199.8 ±19.3 ml/g DM at 24 h incubation and 259.3±18.2 ml/g DM at 48 h incubation.

The results of senage studies from different regions of Bulgaria are presented in figure 3.

From the data in the figure, it can be seen that at 24 h of incubation, the lowest value of gas produced is the senage from farm № 1- 46.3 ml/g DM and the highest value is the alfalfa-wheat senage from farm № 5- 220.9 ml/g DM produced gas.

The lowest value at 48 h incubation is the gas produced by alfalfa senage 32% DM at farm № 4- 93.4 ml/g DM. The highest values are recorded from senage /alfalfa and wheat 35% DM/ from farm № 5- 293.9 ml/g DM. The gas produced at 48 h incubation increased 41.09% compared to 24 h incubation, the variation is almost unchanged. The mean values of the senages, grass masses samples from the eight farms was 115.1 ± 11.07 ml/g DM at 24 h incubation and 162.4 ± 15.6 ml/g DM at 48 h incubation.

From the data of the figure 4 it can be seen that with the lowest values of the produced gases for 24 h of the incubation is the straw at the farm №

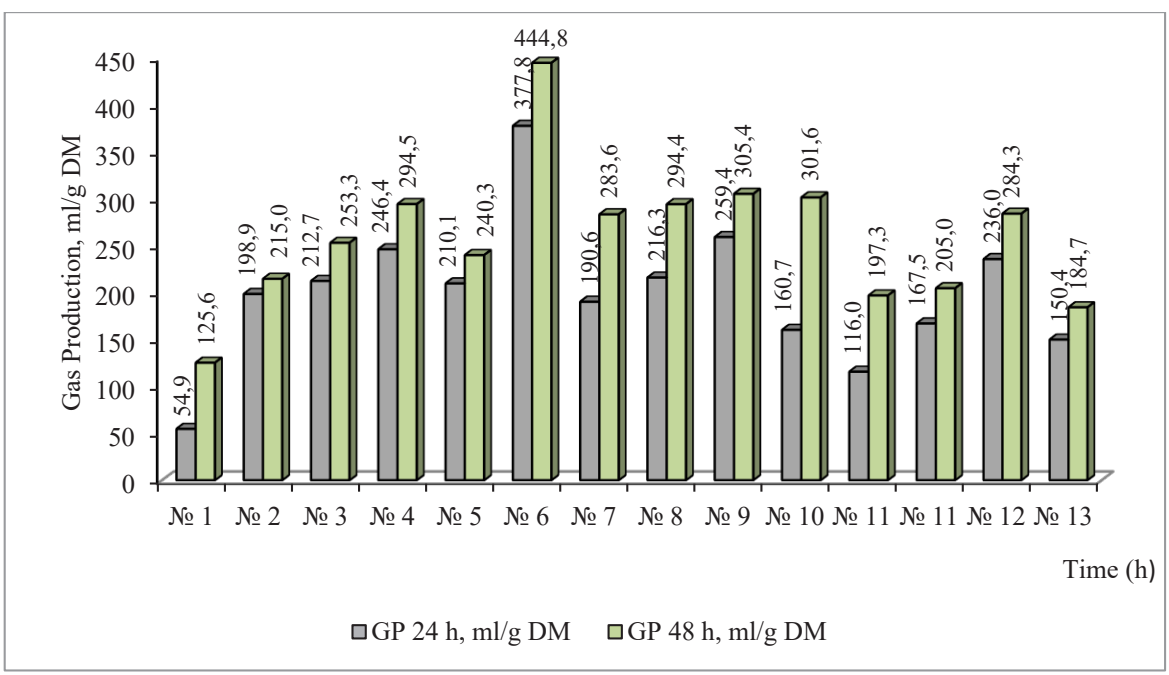

Figure 2. Mean values of gas production GP (ml/g DM) of corn silages from different farms in Bulgaria

10-66.2 ml/g DM, and the highest is the value of the straw at the farm № 7 -266.9 ml/ g DM. The closest to the minimum value is the straw from Farm № 11 with 92.4 ml/g DM separate gas, and the closest to the maximum is the straw sample from Farm № 6 -210.3 ml/g DM. The remaining samples have intermediate values.

Similar values establish Tagliapietra et al., (2011) of wheat straw in "*in vitro*" study at incubation 24 h (GP24) - 149 ± 13 ml/g DM.

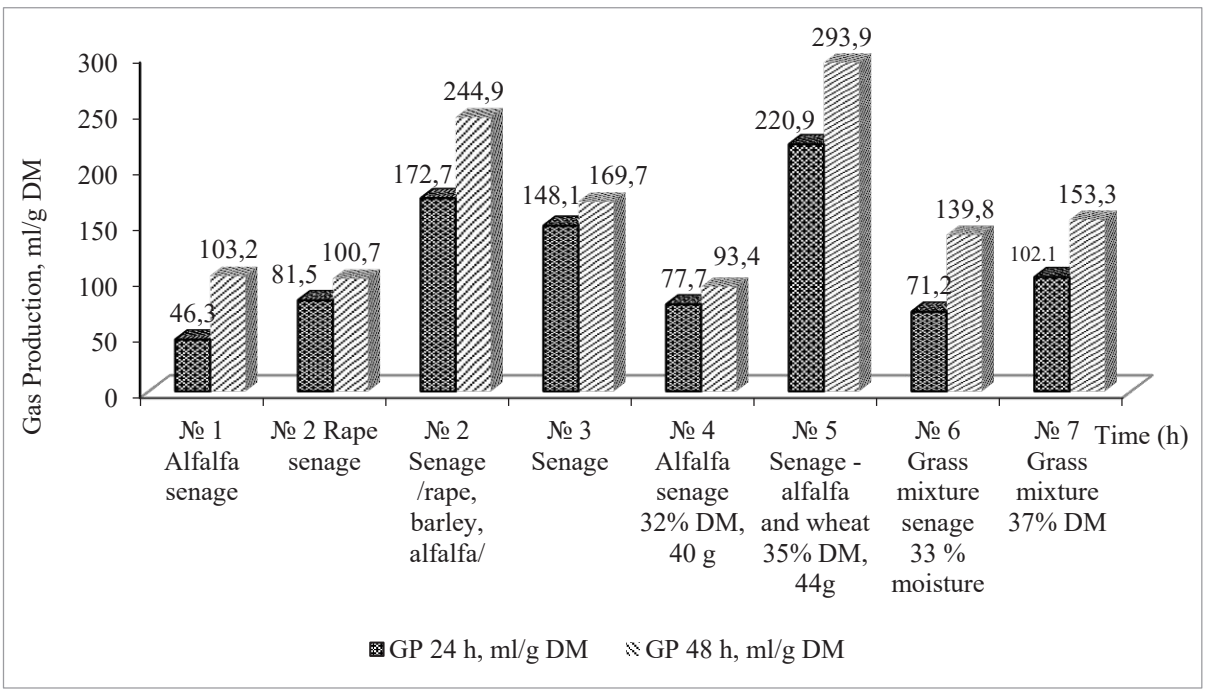

Figure 3. Variation of gas production (GP, ml/g DM) in senages from different farms

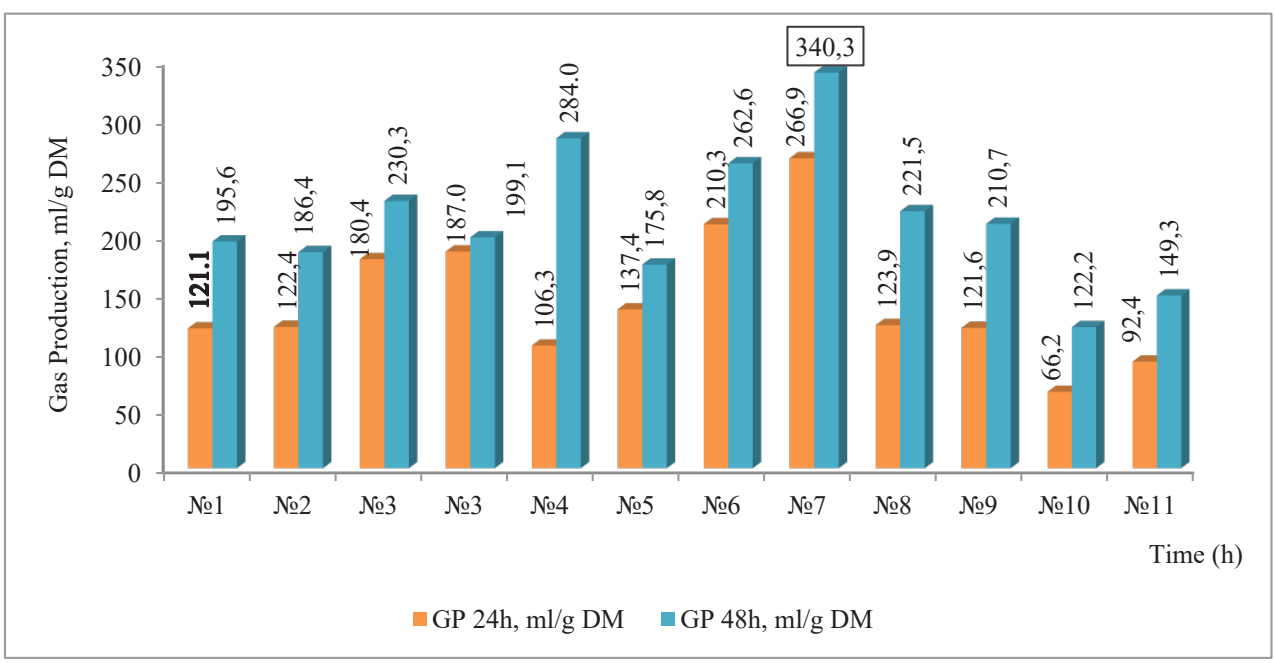

Figure 4. Mean values gas production GP (ml/g DM) of straw from different cow farms in Bulgaria

At 48 h incubation, the highest value is observed in the straw sample from farm № 7 - 340.3 ml/g DM, and the lowest is the value of straw from farm № 10-122.2 ml/g DM.

The mean values of the straw samples from the twelve farms was 144.6 ± 56.2 ml/g DM at 24 h incubation and 205.1 ± 56.6 ml/g DM at 48 h incubation.

Figure 4 reflects the mean gas values the production of straw samples from different regions of the country.

The results of research on alfalfa hay from different areas of the country are shown in figure 5.

The mean values of the alfalfa hay samples from the five farms was 208.3 ± 28.3 ml/g DM at 24 h incubation and 239.9 ± 33.2 ml/g DM at 48 h incubation.

The comparison between the individual feed groups shows that the amount of gas formed during 24 h incubation varies significantly - from 115.1 ml/g DM to 244.5 ml/g DM depending on the specific structure of the feeds in the group. The difference between the lowest and the highest value is more than 2 times. The highest amount of gas is formed from fresh feed - 244.5 ml/g DM. The large amount of gas formed in green fodder is also determined by the rapid decomposition of biomass in the rumen of ruminants due to high digestibility. The second group in terms of volume of gas formed includes – alfalfa hay, silage, straw and haylage. The amount of gas formed in them varies from 115.1 ml/g DM to 208.4 ml/g DM. The smallest amount of gas formed in the second group – haylage, hay, straw, silage is due to the high content of fibers, which decompose more slowly, as well as the fermentation processes that have occurred in the silage and haylage. The results confirm the studies of (Reynolds et al., 2010), (Tamburini et al., 2010) that feed intake, dry matter intake (DMI), type and level of fermentable carbohydrates **(**Ellis et al.*,* 2007) are major parameters affecting the amount of gassing in ruminants.

The amount of gas formed during 48 h incubation is greater for all feeds compared to 24 h incubation. The largest amount of gas formed at 48 h is fresh feeds, silage, hay - 288.4 ml/g DM - 239.9 ml/g DM. Straw and haylage belong to the second group. The amount of gas formed in them varies from 205.1 ml/g DM to 162.4 ml/g DM.

The comparison between the amount of gas formed during 24 h and 48 h incubation shows that during 48 h incubation, the amount of gas formed is on average 21.23% higher. However, the difference in the amount of gas formed during 24 h and 48 h incubation for different feed groups is different. In straw, silage and haylage, the gas formed during 48 h incubation is 29.74%

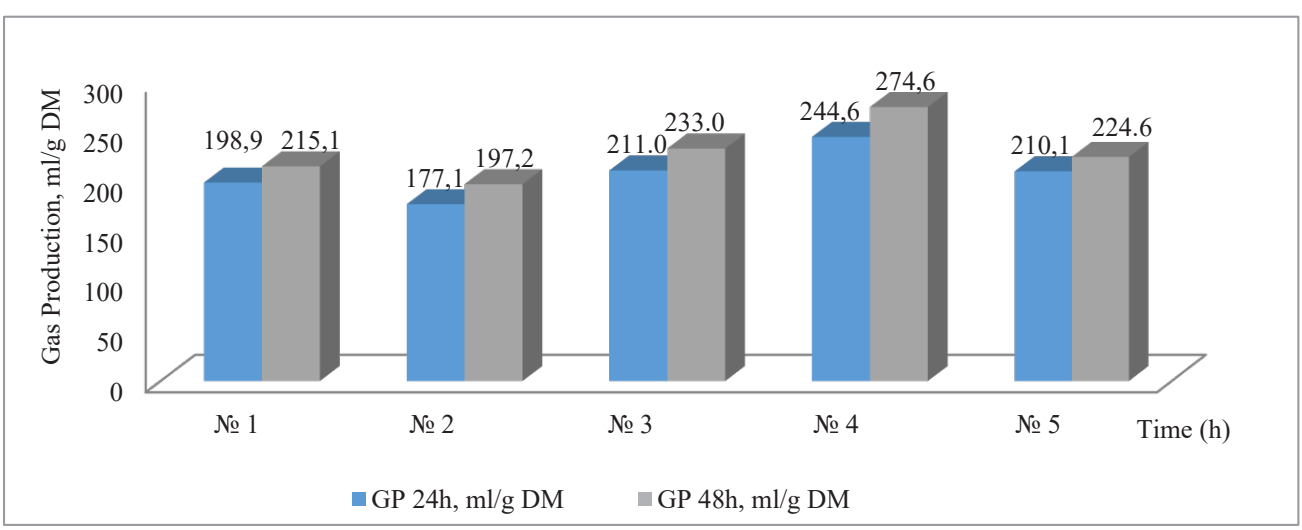

Figure 5. Mean values of gas production GP (ml/g DM) of alfalfa hay

to 41.80% more compared to incubation for 24 h. The second group is formed by fresh feed, haylage, in which the gas formed during 48 h incubation is 15.73% to 21.31% more compared to incubation for 24 h.

The different rate of increase in the amount of gas formed in the cultures during incubation for 24 h and 48 h indicates the different kinetics and rate of decomposition of the substances depending on the composition and structure of the feed. The significantly higher amount of gases formed in straw, silage and haylage during incubation for 48 h shows that in bulky and rough feed, the rate of decomposition is initially slower and accelerates with longer incubation. In fresh feed, haylage, the rate of decomposition and gas formation is initially fast, then slows down.

The analysis of the obtained results allows to draw the following important conclusions.

CONCLUSIONS

According to the amount of gas released, forages can be grouped: fresh forages 244.5 ml/g DM, hay, silage, straw and haylage. The amount of gas formed varies from 115.1 ml/g DM to 208.3 ml/g DM at 24 h incubation. The amount of gas formed at 48 h incubation varied from 288.4 ml/g DM to 162.4 ml/g DM at 48 h incubation.

The mean values of the alfalfa samples from the three farms was 244.5 ± 43.6 ml/g DM at 24 h incubation and 288.4 ± 50.1 ml/g DM at 48 h incubation.

The mean values of the corn silages samples from the fourteen farms was 199.8 ±19.3 ml/g DM at 24 h incubation and 259.3±18.2 ml/g DM at 48 h incubation.

The mean values of the senages, grass masses samples from the eight farms was 115.1 ± 11.07 ml/g DM at 24 h incubation and 162.4 ± 15.6 ml/g DM at 48 h incubation.

The mean values of the straw samples from the twelve farms was 144.6 ± 56.2 ml/g DM at 24 h incubation and 205.1 ± 56.6 ml/g DM at 48 h incubation.

The mean values of the alfalfa hay samples from the five farms was 208.3 ± 28.3 ml/g DM at 24 h incubation and 239.9 ± 33.2 ml/g DM at 48 h incubation.

The amount of gas formed in different types of bulky green forages at 24h and 48h incubation is different and depends on the rate of dry matter decomposition.

In the case of forages - straw, silage and haylage, the gas formed at 48 h incubation was 29.74% to 41.80% more compared to 24 h.

The amount of gas formed as a consequence of fermentation processes in ruminants studied by the "*in vitro"* gas production method depends mainly on the specific chemical composition and structure of the feed.

To reduce the amount of gas produced by ruminants, it is necessary for feeding rations to be well balanced, not only in terms of protein and energy content, but also in terms of structural fiber components. The creation of advanced technological models for feeding productive animals with a balanced participation of bulky feed can contribute to a significant reduction in greenhouse gas emissions in the production of food of animal origin. To develop regulatory and economic regulators stimulating the reduction of greenhouse gas emissions from livestock farming.

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