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Analysis of the Process of Applying Molasses to Grass and Clover Green Fodder in Terms
of Silage Quality for Biogas Plants

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**Abstract:** Silage used in biogas production is one of the main sources of substrate for anaerobic digestion in agricultural biogas plants. However, it is advisable to use plants with high dry matter and sugars content, which can increase biogas efficiency. The study aimed to analyze the effect of selected parameters of the molasses application process to green fodder during its harvesting with a compacting baler on the quality of the obtained haylage. Molasses was applied during the harvesting of semi-dried green fodder in the following variants: before the pick-up and after the pick-up of the round baler in the amount of 5% dry matter. The ensiled raw material was a mixture of red clover and perennial ryegrass, which was characterized by an average dry matter content of 65%. The lowest coefficient of variation of reducing sugar content was found in silage from shredded plants with molasses applied before the cutting unit, which is 27.5%. The bales of green fodder obtained from whole and shredded plants with molasses applied before the cutting unit had the highest average reducing sugars content, respectively: 9.65 g·dm-3 and 9.24 g·dm-3. The quality of haylage was assessed based on the Flieg–Zimmer scale. All analyzed fodders received the maximum number of points and were classified as very good. The highest lactic acid content in the amount of 54.66 g·kg-1d.m was found in haylage from shredded plants with molasses applied before the cutting unit. The pH value of the assessed fodders ranged from 5.43 to 5.53 and was characteristic of preserved fodders with a dry matter content of over 600 g·kg-1d.m. It was found that both the fragmentation of plants and the place of application of molasses to the green forage during harvesting with a round baler affect the amount and distribution of molasses in the bale and the lactic acid content in the haylage.

**Keywords:** biogas, molasses, silage, lactic acid

1. Introduction

Obtaining energy from renewable sources is an important issue in the functioning of each country (Zwolińska & Basta 2024). An important source is biomass, often playing a key role in the transition from energy based on fossil fuels to sustainable energy production (Adelard et al. 2015). Biomass is widely used in energy production, including the production of agricultural biogas. Biogas production is a series of biochemical processes that primarily require organic matter unused by humans and cannot be reused (Ciuła et al. 2023). Agricultural biogas plants use various organic materials as raw material, including energy crops and agricultural residues such as manure and crop residues (Scarlat et al. 2018, Czubaszek et al. 2023). Chemical and biochemical processes leading to biogas formation occur in four basic phases. The first phase is called hydrolysis, in which the complex compounds of the starting material (including carbohydrates, proteins, and fats) are broken down into simple organic compounds (amino acids, sugars, fatty acids). In the next stage, called acidogenesis, the intermediate products formed are broken down by acid-forming bacteria into fatty acids (acetic acid, propionic acid, and butyric acid) and water. Small amounts of lactic acid and carbon dioxide may be formed in this phase. In the next phase, called octogenesis, with the participation of bacteria appropriate for this process, these products are transformed into substances preceding the formation of biogas: acetic acid, hydrogen, and carbon dioxide. Since a hydrogen content that is too high is harmful to acetic bacteria, they must cooperate with methanogenic microorganisms. In the last stage of the biogas formation process (methanogenesis), methanogenic microorganisms consume hydrogen during the formation of methane, creating suitable conditions for the life of acetic bacteria. About 75% of methane is generated from acetates or alcohols. The rest is produced by reducing carbon dioxide with hydrogen (Wiącek & Tys 2017, Onthong & Juntarachat 2017).

To maintain the continuity of substrate supplies to biogas plants, they are often preserved by ensiling. Ensiling is one of the basic preservation methods, consisting of acidifying the ensiled material with lactic acid produced from bacterial fermentation of sugars (Sun et al. 2021). Factors influencing the correct course of fermentation include quality of the raw material, method of collection, temperature, anaerobic conditions, purity of the ensiled material, and appropriate ensiling technique (Chachułowa et al. 1997, Dulcet 2001, Holzer et al. 2003). With the appropriate concentration of lactic acid and low pH, silage does not spoil and can be stored for a long time without access to air. At the same time, efforts are made to inactivate bacteria, fungi, and plant enzymes involved in the decomposition of proteins or the production of undesirable substances (Rotz 2003, Jeroch & Lipiec 2012, Soukup 2012). The most common cause of poor-quality silage is the lack of readily available sugar necessary to produce the required amounts of lactic acid. Poor silage is unsuitable for biogas production because its quality determines the amount of gas-containing methane obtained (Węglarzy & Podkówka 2010). The fermentation process for obtaining very good quality silage using various preparations is currently one of the most dynamically developing energy-saving methods of their production. Their effectiveness in the process of silage of green fodder depends on the even mixing of a precisely defined amount of them with the plant material. One of the preparations that improve silage quality is molasses, i.e., the final product of crystallization of the physical process of sugar extraction. It contains 80% dry matter, 35% sucrose, 15% glucose, and 4.5% nitrogen compounds. Molasses is used as an additive to silage due to the content of soluble carbohydrates and is also often used in concentrations of 4-5% in silage from legumes and legumes (Anaya-Reza & López-Arenas 2018). In the case of using molasses as an additive for silage, there is a noticeable lack of description and influence of basic factors determining the technique of its application to green fodder. The difficulties related to molasses application have already been pointed out by Baytok et al. (2005), Podkówka (1979), and Dulcet (2001). The research results published so far mainly concern the influence of molasses on the quality of obtained silage (Chen et al. 2014, Qamar 2009, Hashemzadeh-Cigari et al. 2011, Petit & Veira 1994, Leibensperger et al. 1988, Tjandraatmadja et al. 1994), and the few studies on molasses application covered technologies of green fodder harvesting using a field chopper (Pieper et al. 2006). Lack of knowledge on this subject prevents the development of an effective technique for applying molasses to green fodder during its harvest, which would ensure the effective action of molasses in the process of green fodder ensiling and consequently obtaining a high-quality product. The gaps in the subject mean that the valuable raw material molasses is rarely used (Dulcet 2005).

According to many researchers, increasing the density of green fodder in bales and its cutting positively affect the fermentation process (Sun et al. 2010, Keller et al. 1997, Muck et al. 2004, Fuerll et al. 2008). Waszkiewicz (2004) states that shorter, uniform particles of plant material ensure a uniform course of the lactic acid fermentation process in the entire heap volume. The silage quality intended for biogas production is a key factor influencing the efficiency of the anaerobic digestion process and biogas yield. Preserving grass in the form of silage is preferred in biogas plants because silage guarantees high quality and a constant supply of raw material (Nizami et al. 2009, Czubaszek et al. 2022). According to studies, grass silage usually has a higher CH4 yield than fresh biomass. It can be stored for a long time and, therefore, used all year round (Lehtomäki et al. 2008, Ambye-Jensen, et al. 2013, Zhao et al. 2017, Gallegos et al. 2017).

The study aimed to analyse the influence of selected parameters of the molasses application process to green fodder during its harvesting with a round baler on the quality of the obtained haylage, which can be used for biogas production.

2. Materials and Methods

2.1. Object of research

The study used a mixture of red clover (*Trifolium pratense L.)* and perennial ryegrass (*Lolium perenne* *L*.). The plant mixture came from a farm using conventional cultivation methods. The plants were mowed in the clover flowering phase (1st cut) and then dried. The average green mass yield for the 1st cut was
28 t∙ha-1. The dominant plant in the mixture was red clover and constituted approx. 60% of the plant mass. The dry mass yield was obtained at the level of 7.6 t·ha-1. The green fodder was dried in the field to 65% DM. The moisture content of the dried green fodder was determined using a dryer scale. To facilitate application, the molasses was diluted with water in a mass ratio of 7:1 (7 kg of molasses and 1 kg of water). Based on the analysis of the literature and own research, the TeeJet TK-10 impact nozzle was used for the study. The non-uniformity index of the molasses solution dosing for the nozzle used in the study did not exceed 7%. The bales were rolled using a Sipma Z-590/1 Power Cut baler with a cutting width of 1.2 m and a theoretical cutting length of 0.1 m. The average length of the plants before and after cutting was calculated using the formula (Kanafojski 1980):

$l\_{m}=\frac{\sum\_{i=1}^{i=n}\overbar{x}∙n\_{i}}{100} \left[mm\right]$ (1)

where:

lm – the average length of plants for all classes [mm],

i – number of classes,

n – final class number,

$\overbar{x}$ − average length of plants in individual classes [mm],

ni – number of sections of the average length of plants in a given class.

Molasses was applied in the amount of 5% of the dry mass of the green fodder. Table 1 presents the application parameters of the molasses solution obtained experimentally and used in the actual field tests. Table 2 presents the properties of the applied molasses.

**Table 1.** Molasses application parameters

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nozzle type | Nozzle working width s [m] | Nozzle distance from the green fodder [m] | Pressure [MPa] | Molasses dilution with water |
| TK 10 | 1.2 | 0.32 | 0.15 | 7 : 1 |

**Table 2.** Physicochemical properties of molasses

|  |  |
| --- | --- |
| Feature | Wartość |
| Dry matter content [%], | 76 |
| Density [g∙cm-3] | 1.36 |
| Dynamic viscosity [Pa∙s]∙10-3(depending on temperature) | 600 (32°C) |
| Sucrose content [%], | 36 |
| PH value | 7.2 |

Molasses is particularly beneficial when applied to crops low in soluble carbohydrates and high in protein (Keskin & Yilmaz 2005). The addition of molasses to low DM forage may result in a loss of carbohydrates during silage run-off during the first few days (Titterton & Bareeba 1999).

2.2. Methodology of the study

Molasses was dosed during the harvest of green fodder in the following variants (Fig. 1):

* W 1 – harvest of whole plants without the addition of molasses (control), lm = 453 mm,
* W 2 – harvest of whole plants with the addition of molasses applied before the pick-up onto the green fodder roller, lm = 453 mm,
* W 3 – harvest of whole plants with the addition of molasses applied before the cutting unit,
lm = 453 mm
* W 4 – harvest of green fodder with crushing and with the addition of molasses fed before the pick-up,
lm = 178 mm,
* W 5 – harvest of green fodder with crushing and adding molasses fed before the cutting unit, lm = 178 mm.



Fig. 1. Applicator nozzle attachment points on the Sipma Z – 590/1 round baler:

1 – forage roller, 2 – tractor, 3 – applicator, 4 – round baler, I – applicator nozzle placed in front of the round baler cutting unit, II – applicator nozzle placed behind the pick-up/in front of the round baler cutting unit

The mixing of molasses with dried green fodder was characterized using the mixing unevenness index (coefficient of variation) of reducing sugars contained in the collected samples:

$K=\frac{φ}{x\_{śr}}∙100\% $ (2)

where:

K – mixing non-uniformity index (variation coefficient) for reducing sugars [%],

ϕ – standard deviation,

xśr – arithmetic mean sugar content [g·dm-3].

The sampling locations to determine the molasses distribution in the bale are shown in Fig. 2.

**0.2 m 0.3 m 0.3 m**

 **0,6 m 0.3 m**

**∅ 1.2 m**

**A**

**B**

**C**

**1.2 m**

 **1 2 3**

 **1 2 3**

 **1 2 3**

**∅ 0.1 m**

**Fig. 2.** Sampling locations to determine molasses distribution in the bale cross-section and silage quality

The content and distribution of molasses in the bales were determined for two positions of the applicator nozzle on the baler. The tests were conducted in field conditions. The same application parameters were used in each harvest variant. Immediately after the dried green fodder was harvested, samples were taken for laboratory analysis. The content and distribution of molasses in the cross-section of the bales were determined based on the content of reducing sugars in the samples taken. The test material was taken from five bales for each harvest variant. The determination was performed using the colorimetric method, using the reducing properties of sugars, which in an alkaline environment reduce the nitro groups of 3,5-dinitrosalicylic acid (DNS) to amine groups characterized by an orange color. The intensity of the color, proportional to the content of reducing sugars, is the basis for their quantitative determination (Ghose 1987).

After harvesting, the bales were wrapped four times in foil and stored indoors at a temperature of 18°C. The bales were wrapped with a self-adhesive stretch foil for silage, white, 500 mm wide, and 0.025 mm thick. The haylage quality was assessed 6 weeks after ensiling. Samples of equal weight were taken from the bale according to the scheme presented in Fig. 2. The quality of the haylage was assessed based on the content of lactic, butyric, and acetic acids, using the Flieg-Zimmer scale (Jamróz et al. 2001). The Lepper method was used to quantify the content of acids. In addition to the content of fatty acids, the following were determined in each of the haylage samples:

* dry matter – PN**-**ISO 6496,
* crude ash – PN-76/R-64-795,
* total protein – PN-EN ISO 5983-1,
* raw fiber – PN-EN ISO 6865,
* pH – pH meter N 5172.

The content of dry matter, crude ash, total protein, and crude fiber was determined following Commission Regulation (EC) No 152/2009. Chemical analyses were performed in five replicates for each set variant. The test results were subjected to variance analysis. Null hypotheses were verified using the F test at a significance level of 0.05. The significance of differences between the experimental groups was assessed using the Tukey test. The measurements were performed using the same measuring devices and the same measurement procedures.

3. Results and Discussion

3.1. Content of reducing sugars

To determine the application parameters, it was necessary first to determine the bale density in relation to dry mass. The average values of plant material density in bales obtained during the tests are presented in Table 3.

**Table 3.** Average values of forage density of different lengths

|  |  |
| --- | --- |
| DM content [%] | Bale density [kg·d.m·m-3] |
| Material not cut lm = 453 mm | Material cut lm = 178 mm |
| Baling pressurepp = 18 MPa | Baling pressurepp = 18 MPa |
| 65 | 138 | 155 |

Based on the analysis of the plant material density, it was found that the density of bales formed from plants cut with the cutting unit of the round baler is about 12% higher in comparison with bales formed from uncut plants (Tab. 3). The obtained amounts of reducing sugars in individual bales and the coefficients of variation are presented in Table 4.

**Table 4.** The content of reducing sugars determined in bales

|  |  |  |  |
| --- | --- | --- | --- |
| Bale type | Average reducing sugar content in bales [g·dm-3] | Standard deviation | Mixing non-uniformity index (variation coefficient) for reducing sugars K [%] |
| W 1 | 2.641 | 0.547 | 20.7 |
| W 2 | 7.392 | 4.512 | 61.0 |
| W 3 | 9.649 | 4.635 | 48.0 |
| W 4 | 7.930 | 2.978 | 37.6 |
| W 5 | 9.240 | 2.545 | 27.5 |

The graph of the content of reducing sugars obtained in the individual harvest variants is presented in Figure 3. Based on the coefficient of variation K, it can be stated that the plant material was heterogeneous in terms of the content of reducing sugars in the places where samples were taken from the bale. The coefficient of variation for the control bales (harvest variant W 1) was 20.7% and was the lowest compared to the other bales. Analyzing the bales to which molasses was applied during harvesting with a round baler, the highest coefficient K was recorded in bales from variant W 2 (with uncrushed material, molasses applied before the pick-up onto the green forage roller), which was 61%. A clear reduction in the unevenness of mixing molasses with green forage was obtained for bales from variants W 3 and W 4 – 48% and 37.6%, respectively. The lowest coefficient of sugar content variation in green forage at 27.5% was obtained in bales of variant W 5 (shredded green forage, molasses applied before the cutting unit). Applying additives to a roller or swath causes the preparation to be evenly mixed with the green fodder under the influence of the moving elements of the harvesting machines, and the process of ensilaging the green fodder begins already in the machine (Dulcet 1998, Dulcet 2010, Roszkowski 1979).

**Harvest variant**

Fig. 3. The average reducing sugar content in the collected green fodder samples depending on the harvest variant.

Lowercase letters indicate statistical differences (p ≤ 0.05)

3.2. Analysis of the quality and chemical composition of the obtained silage

The pH value of the tested haylages was at the level of 5.43-5.53 and was characteristic of preserved feeds with a high dry matter content of over 60% (McDonald et al. 1991). The differences were not statistically significant (p ≤ 0.05).

All tested haylages subjected to quality assessment according to the Flieg-Zimmer scale obtained the maximum number of points – 100, which allows them to be classified as very good. Due to the variability of the lactic acid content in the haylages, this parameter was subjected to statistical analysis. The highest lactic acid content of 54.66 g·kg-1d.m was found in the haylage made from shredded plants with molasses applied before the cutting unit (Fig. 4). This silage differed statistically significantly from the other variants (p ≤ 0.05). The lowest level of this acid was found in the control silage (without added molasses) – 29.82 g·kg-1d.m. Acetic acid content did not differ statistically significantly in the feeds considered (p ≤ 0.05).

No butyric acid was found in the analyzed silage variants, indicating that the anaerobic digestion process was in the right direction. In the studies conducted by Tjandraatmadjai et al. (1994), adding molasses in the range of 4% to 8% increased the content of lactic acid, while the amount of other fatty acids decreased.

The crude ash content (Fig. 5) ranged from 68.5 g·kg-1d.m (for the harvest variant W 5) to 90.3 g·kg-1d.m (for the control silage W 1). These values indicate that the silage was not contaminated with soil because the permissible crude ash content in good silages should not exceed 10% of dry matter (McDonald et al. 1991). The highest total protein content (Fig. 6) was found in silages from chopped plant mass with the addition of molasses (W 4, W 5) – 181.9 g·kg-1d.m and 179.7 g·kg-1d.m, respectively. These values differed significantly from the results obtained in the remaining silages, which contained less of this nutrient (p ≤ 0.05). The concentration of crude fiber (Fig. 7) in the analyzed silages did not exceed g·kg-1d.m. It was the highest in the control silage and the lowest in the silage made from chopped biomass with molasses applied before the cutting unit (harvest variant W 5) and in the silage made from uncut raw material with molasses applied before the cutting unit (harvest variant W 3). As reported by (Schattauer & Weiland 2005), silages characterized by a lower content of crude fiber are a better substrate for biogas production.

**lactic acid**

**acetic acid**

**Fig. 4.** The average content of lactic and acetic acid content in silage obtained from individual forage harvest variants.

Lowercase letters indicate statistical differences (p ≤ 0.05)

Fig. 5. The average content of crude ash content (CA) in silage obtained from individual forage harvest variants.

Lowercase letters indicate statistical differences (p ≤ 0.05)

Fig. 6. The average content of total protein (TP) content in silage obtained from individual forage harvest variants.

Lowercase letters indicate statistical differences (p ≤ 0.05)

Fig. 7. The average content of raw fiber content (FC) in silage obtained from individual forage harvesting variants.

Lowercase letters indicate statistical differences

4. Conclusion

As a result of the research and analyses conducted, it was found that the harvest parameters of dried green fodder and the addition of molasses allow for the obtaining of silage of good quality and high lactic acid content. The process of applying molasses to green fodder collected with a baler at this stage, analyzed in the work, can be implemented in practice. Still, it requires continuous improvement and further research in this area, as also written by other authors (Dulcet 2001, Dulcet & Kwiatkowski 2002, Chen et al. 2014, Jamróz et al. 2001, Pieper et al. 2006). Based on the analysis of the obtained results, the following conclusions can be drawn:

1. The highest lactic acid content was characterized by silage from shredded plants with molasses applied before the cutting unit.

2. In the case of reducing sugars, the highest amount was found in bales of green fodder obtained from whole and shredded plants with molasses applied before the cutting unit. Based on these values, it can be concluded that the amount of reducing sugars in the bales is influenced by the place where the molasses is applied.

3. The lowest value of the coefficient of variation of the reducing sugar content was obtained for bales of green fodder from shredded plants with molasses applied before the cutting unit.

4. Shredding the plants significantly increased the content of total protein (BO).

5. Based on the conducted research and analyses, it can be concluded that the place where the molasses is applied and the shredding of the plants during the harvest of green fodder with a round baler significantly affect the content of lactic acid.

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