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# EFFECT OF VARIOUS ADDITIVES ON THE CHEMICAL COMPOSITION, FERMENTATION PARAMETERS AND APPARENT DIGESTIBILITY OF VIRGINIA FANPETALS SILAGE IN SHEEP*

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## Abstract

The aim of this study was to determine the effect of various additives (straw, grass, molasses, dried sugar beet pulp – pellets, inoculant, organic acids) on the quality and fermentation profile of Virginia fanpetals (*Sida hermaphrodita* L.) silage. Silage samples were assayed for the content of dry matter – DM, crude ash, crude protein – CP, water soluble carbohydrates – WSC, neutral detergent fiber – NDF, acid detergent fiber – ADF and acid detergent lignin – ADL as well as pH and the concentrations of ammonia nitrogen, lactic acid, acetic acid and butyric acid. Silage quality was evaluated according to the DLG Key. Apparent digestibility was determined in sheep, and the digestibility coefficients of DM, CP, NDF, organic matter – OM and digestible OM were calculated. The tested additives increased DM, decreased ADL content ($P \leq 0.01$), and reduced WSC utilization ($P \leq 0.05$, $P \leq 0.01$) in silages. Only sugar beet pulp decreased the content of NDF and ADF ($P \leq 0.01$). All silages had similar pH values (4.01 - 4.10). The analyzed silages were characterized by intense lactic acid fermentation, which was inhibited only by the grass additive ($P \leq 0.01$). All additives suppressed acetic acid fermentation ($P \leq 0.05$, $P \leq 0.01$). Grass and sugar beet pulp significantly decreased the concentration of butyric acid ($P \leq 0.01$). The addition of grass decreased CP digestibility (from 92.1% in the control silage to 89.3%), whereas the addition of a bacterial inoculant ($P \leq 0.01$) and a blend of organic acids ($P \leq 0.05$) increased CP digestibility to 93.8% and 92.8%, respectively. The addition of grass and sugar beet pulp increased the coefficient of NDF digestibility by 6 percentage points relative to 60.9% in the control silage. The addition of the inoculant and organic acids decreased NDF digestibility to 56.0% and 50.5%, respectively. Dried sugar beet pulp was the most effective additive, which positively affected the chemical composition, fermentation profile and apparent digestibility of *Sida* silage.

**Keywords:** Virginia fanpetals, silage additive, apparent digestibility.

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# INTRODUCTION

Maize silage, supplemented with grass silage or alfalfa silage, is the major type of feed offered to ruminants (Purwin et al. 2009). The constantly increasing production costs have spurred interest in alternative plant protein sources (Antoszkiewicz et al. 2019). One of alternative protein crops in ruminant nutrition could be Virginia fanpetals (*Sida hermaphrodita* (L.) Rusby), a perennial plant of the family *Malvaceae*, with a productive life of 15 - 20 years. The species is native to North America, and it was introduced to Poland in the mid-20$^{th}$ century, as a valuable source of fiber and biomass for renewable energy production. Virginia fanpetals is also a herbaceous plant and a source of nectar. *Sida* can be combusted in the form of chips and pellets (Borkowska, Molas 2012). Due to its high yield potential, *Sida* biomass is also a valuable substrate for biogas production (Jablonowski et al. 2017).

Virginia fanpetals is a cold-hardy and drought tolerant plant, it is resistant to lodging and moderately resistant to pests and diseases, and has low soil requirements (Žavoronkova 2010, Franzaring et al. 2014, Dudek et al. 2017). *Sida* biomass has high utilization potential, and it can be used as a roughage source in ruminant diets. Previous studies have focused on the chemical composition of Virginia fanpetals grown at different fertilization levels and harvested in different growth stages. The efficiency of dried Virginia fanpetals herbage in ruminant nutrition has also been analyzed (Tarkowski 2006). *Sida* biomass harvested in the early bud formation stage is a good source of crude protein (approx. 200 g kg$^{-1}$ DM), therefore it can be fed not only to ruminants but also to pigs and rabbits (Borkowska 1991, Tarkowski 2006).

Virginia fanpetals and alfalfa have similar chemical composition (Giwa 2017). Leaves, which account for approximately 50% of total biomass, contain considerable amounts of vitamin C, carotene and lipids. *Sida* has also a high content of calcium and phosphorus. A few studies conducted to date revealed that milk from cows fed protein-fiber extrudate (dried *Sida* herbage and faba bean meal) had a higher content of fat and protein than milk from cows fed standard concentrates, maize silage and meadow hay (Tarkowski 2006).

During biomass ensiling, the main causes of energy losses are aerobic respiration, fermentation, aerobic decomposition and heat generation (Davies et al. 2005). The ensiling process should be preceded by structural damage to stems during harvest. *Sida* silage analyzed in a previous study (Fijałkowska et al. 2017) was characterized by a fast rate of lactic acid fermentation and low concentrations of volatile fatty acids.

The aim of this study was to determine the effect of various additives on the quality and fermentation profile of Virginia fanpetals silage based on its chemical composition, fermentation parameters and apparent digestibility in sheep.

# MATERIALS AND METHODS

The analyzed plant material was Virginia fanpetals herbage and silage. First-cut green biomass was harvested in the bud formation stage (on 11 June 2015) with a forage harvester, on a commercial plantation in north-eastern Poland. At the beginning of the growing season, the plantation was fertilized with 100 kg N ha$^{-1}$, 50 kg $K_2O$ ha$^{-1}$ and 80 kg $P_2O_5$ ha$^{-1}$. Herbage samples were collected after harvest ($n$=3). *Sida* biomass was ensiled in 220 L standard open-head high-density polyethylene (HDPE) drums (Brenntag GmbH, Germany) without drainage holes. The following types of silage were made: CO – control, without additives; SS – with the addition of straw (2 kg); SG – with the addition of grass (1:1), SM – with the addition of molasses (30 g kg$^{-1}$ fresh matter (FM)); SB – with the addition of dried sugar beet pulp pellets (9:1); SI – with the addition of a bacterial inoculant (*Lactobacillus plantarum, Lactobacillus buchneri, Lactococcus lactis*), $2.5 \cdot 10^8$ CFU kg$^{-1}$ FM; SA – with the addition of a blend of organic acids (propionic acid, formic acid, lactic acid, ammonium formate, ammonium propionate), 3 g kg$^{-1}$ FM. All additives were used in the recalculate of FM of herbage. Addition of straw as a drainage and juice absorbing layer in 2 kg 100 kg$^{-1}$ FM; absorbent additives (wilted ryegrass and dried beet pulp pellets) were mixed in 1:1 and 9:1 FM proportions, respectively, to obtain DM value guaranteeing no leakage, aiming at a DM of 27%. The addition of molasses was established in previous laboratory studies. In the SI and SA variants, the additives were used in the lowest doses recommended by the manufacturer. After 90 days, silage samples ($n$=3) were collected with a probe (80 mm in diameter) along the entire length of the drums. Herbage and silage samples were dried at 60°C in a Binder FED 115 dryer, and ground in a Retsch SK 100 mill to a 1 mm particle size.

The samples were assayed for the content of dry matter (DM), crude ash and crude protein (CP) by standard methods described in Purwin et al. (2009), water soluble carbohydrate (WSC) by the anthrone method (Thomas 1977), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by the method proposed by Van Soest et al. (1991). The concentrations of lactic acid, acetic acid and butyric acid were determined in silage as described by Kostulak-Zielińska, Potkański (2001) and Gąsior (2002). Acetic acid and butyric acid were separated and determined by gas chromatography on a Varian 450 gas chromatograph with the Varian CP-8410 autosampler. Lactic acid content was determined by high performance liquid chromatography (HPLC, SHIMADZU) with isocratic flow. The ammonia nitrogen content of silage was determined by the method described in Purwin et al. (2009), and acidity was measured with the HI 8314 pH-meter (Hanna Instruments, USA). Silage quality was assessed according to the DLG Key (Weissbach, Honig 1992).

The apparent digestibility of nutrients was determined by the balance method in four adult meat-type rams kept in individual metabolism cages with fecal collection bags and free access to water. The adjustment period lasted for 14 days. During the 5-day experimental period proper, silage was the only forage, and it was offered *ad libitum* twice daily. Feces and leftovers were collected for 5 days. The samples were frozen. After thawing, feces samples from each ram were used to prepare a bulk sample. The samples were analyzed for the content of DM, ash, CP (Purwin et al. 2009) and NDF (Van Soest et al. 1991).

Based on the results of assays of silages, the net lactation energy (NEL) was calculated in the Feeding Program (MAX System for Dairy, System DLG). The results were processed statistically by one-way analysis of variance (ANOVA). The significance of differences between means was estimated with the Duncan's test. Mean values and the standard error of the mean (SEM) were calculated. All calculations were performed with the use of Statistica v. 12.0 software.

## RESULTS AND DISCUSSION

The DM content of Virginia fanpetals herbage was higher than that reported by Tarkowski (2006), due to low precipitation levels during the growing season and harvest. The higher DM content had a beneficial influence on fermentation in the control silage (Table 1). Except for a blend of organic acids, the remaining additives significantly ($P \leq 0.01$) increased the DM content of silage, relative to the control silage (Table 2). The *Sida* silages produced in this experiment had similar CP content to the alfalfa silage analyzed by Purwin et al. (2015) and Broderick (2004). All types of *Sida* silage and higher CP content than red clover silage (153 g kg$^{-1}$ DM) – Purwin et al. (2015), excluding the silage with the grass additive, where CP concentration was significantly ($P \leq 0.05$) lower. The CP content of silages with the addition of straw, molasses and sugar beet pulp was similar to the CP content of ryegrass silage (184 g kg$^{-1}$ DM) in a study by Broderick et al. (2002). Silages with the addition of a bacterial inoculant or a blend of organic acids had the highest CP content. Alves et al. (2011) also noted an increase (by 75 g kg$^{-1}$ DM) in the CP content of ryegrass silage made with the addition of a bacterial inoculant. Rowghani and Zamiri (2009) observed an increase in the CP content of corn silage made with the addition of a microbial inoculant and formic acid. In the present study, the tested additives significantly reduced the utilization of WSC by microorganisms (by 40% in the variant with grass). The concentrations of WSC were significantly higher in the silage with the addition of a bacterial inoculant ($P \leq 0.05$) and in silages containing straw, grass, molasses and sugar beet pulp as additives ($P \leq 0.01$), compared with the control silage. Purwin et al. (2010) also demonstrated that additives affected the WSC content of silage during fermentation. According

Table 1

Fermentation parameters (g $kg^{-1}$ DM) of Virginia fanpetals silage

| Silage | pH | $N\text{-}NH_3$ (g $kg^{-1}$) TN | Lactic acid | Acetic acid | Butyric acid | Chemical assessment of silage quality according to the DLG Key (points) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | butyric acid | acetic acid | $N\text{-}NH_3$/TN | pH/DM | total | quality |
| CO | 4.08 | $110^{A}$ | $125^{A}$ | $21.4^{Aa}$ | $9.29^{A}$ | 30 | 0 | 20 | 25 | 75 | high |
| SS | 4.07 | $73.6^{B}$ | 109 | $17.6^{B}$ | 7.84 | 30 | 0 | 25 | 25 | 80 | high |
| SG | 4.10 | $48.4^{B}$ | $86.0^{B}$ | $14.4^{B}$ | $4.73^{B}$ | 40 | 0 | 25 | 25 | 90 | high |
| SM | 4.05 | $38.8^{B}$ | 107 | $17.1^{B}$ | 10.0 | 30 | 0 | 25 | 25 | 80 | high |
| SB | 4.05 | $42.9^{B}$ | 108 | $15.0^{B}$ | $3.40^{B}$ | 45 | 0 | 25 | 25 | 95 | very high |
| SI | 4.01 | $50.1^{B}$ | 112 | $18.6^{b}$ | 8.21 | 30 | 0 | 25 | 25 | 80 | high |
| SA | 4.05 | $80.0^{B}$ | 121 | $17.4^{B}$ | 9.03 | 30 | 0 | 25 | 20 | 75 | high |
| SEM | 0.02 | 4.90 | 2.77 | 0.48 | 0.50 | na | na | na | na | na | na |

DM – dry matter, TN – total nitrogen, na – not applicable, SEM – standard error of the mean, significance levels: *a*, *b* – $P \leq 0.05$, *A*, *B* – $P \leq 0.01$ relative to the control silage.

Silage types: CO – control, without additives, SS – with the addition of straw (2 kg), SG – with the addition of grass (1:1), SM – with the addition of molasses (30 g $kg^{-1}$ FM), SB – with the addition of dried sugar beet pulp pellets (9:1), SI – with the addition of a bacterial inoculant: *Lactobacillus plantarum, Lactobacillus buchneri, Lactococcus lactis* ($2.5 \cdot 10^{8}$ CFU $kg^{-1}$ FM), SA – with the addition of a blend of organic acids.

Table 2

Chemical composition of Virginia fanpetals herbage and silage (g kg$^{-1}$ DM)

| Item | DM (g kg$^{-1}$) | Ash | CP | WSC | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|
| Herbage | $197^{B}$ | $83.5^{Aa}$ | $199^{a}$ | $73{,}0^{Aa}$ | $403^{A}$ | $308^{A}$ | $38.0^{B}$ |
| Silage | | | | | | | |
| CO | 160 | 86.0 | 193 | 6.00 | 423 | 371 | 61.0 |
| SS | $213^{A}$ | 81.0 | 175 | $23.0^{B}$ | 394 | 339 | 56.0 |
| SG | $256^{A}$ | 94.0 | $138^{b}$ | $45.0^{B}$ | 472 | 339 | $52.0^{A}$ |
| SM | $214^{A}$ | $75.0^{b}$ | 181 | $15.0^{B}$ | 336 | 305 | $55.0^{A}$ |
| SB | $245^{A}$ | $64.0^{B}$ | 166 | $12.0^{B}$ | $245^{B}$ | $199^{B}$ | $40.0^{A}$ |
| SI | $205^{A}$ | 85.0 | 207 | $10.0^{b}$ | 369 | 303 | $55.0^{A}$ |
| SA | 179 | 81.0 | 201 | 12.0 | 365 | 330 | 50.1 |
| SEM | 13.1 | 3.11 | 7.72 | 4.37 | 23.4 | 18.5 | 2.27 |

DM – dry matter, Ash – crude ash, CP – crude protein, WSC – water soluble carbohydrate, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin, SEM – standard error of the mean, significance levels: *a*, *b* – $P \leq 0.05$, *A*, *B* – $P \leq 0.01$ relative to the control silage.

Silage types: CO – control, without additives; SS – with the addition of straw (2 kg), SG – with the addition of grass (1:1), SM – with the addition of molasses (30 g kg$^{-1}$ FM), SB – with the addition of dried sugar beet pulp pellets (9:1), SI – with the addition of a bacterial inoculant: *Lactobacillus plantarum, Lactobacillus buchneri, Lactococcus lactis* ($2.5 \cdot 10^{8}$ CFU kg$^{-1}$ FM), SA – with the addition of a blend of organic acids.

to Purwin et al. (2009), the addition of inoculants contributed to higher utilization of WSC during ensiling, which was also observed in this study. The analyzed *Sida* silages had considerably lower NDF content and similar ADF content, compared with alfalfa silage in the experiments performed by Broderick (2004) and Broderick et al. (2002), which were determined at 451 and 435 g kg$^{-1}$ DM (NDF), 352 and 347 g kg$^{-1}$ DM (ADF), respectively. The ADL content of the analyzed *Sida* silages was comparable with the ADL content of grass and alfalfa silage (58.1 - 43.9 g kg$^{-1}$ DM) in a study by Purwin et al. (2009). The addition of grass, molasses, sugar beet pulp and a bacterial inoculant ($P \leq 0.01$) decreased the content of the indigestible ADL fraction in experimental silages, relative to the control silage. The addition of sugar beet pulp significantly ($P \leq 0.01$) decreased the content of NDF and ADF in silage. This can be attributed to the fact that the NDF of sugar beet pulp has a high content of hemicelluloses that are readily hydrolyzed in an acidic environment. Rezaei et al. (2009) also found that the NDF content of ensiled amaranth decreased most probably due to cell wall degradation by plant cellulolytic enzymes or acid hydrolysis.

According to previous research (Tarkowski 2006), Virginia fanpetals ensiled without additives was characterized by poor quality, which was significantly improved (to very high quality) when molasses was added. In the present study, all evaluated silages were characterized by intensive lactic acid fermentation and received high and very high (the variant with sugar beet pulp) quality scores according to the DLG Key (WEISSBACH, HONIG 1992). Only the grass additive (1:1) significantly ($P \leq 0.01$) decreased the content of lactic acid relative to the control silage. Acetic acid fermentation was significantly ($P \leq 0.05$, $P \leq 0.01$) suppressed in all types of silage with additives, and butyric acid content decreased ($P \leq 0.01$) in silages with the addition of grass and sugar beet pulp (Table 1). The decrease in the concentrations of acids in silages with additives resulted from an increase in DM content and changes in osmotic pressure, which first inhibit the growth of *Clostridium* spores and enterobacteria responsible for acetic acid fermentation, and then lactic acid bacteria (PURWIN et al. 2009). PURWIN et al. (2009) reported similar concentrations of fermentation end products in maize, grass and alfalfa silages, pointing to their very high quality. A positive effect of a microbial inoculant and formic acid on the concentration of lactic acid, accompanied by a decrease in the levels of acetic acid and butyric acid, was also observed by ROWGHANI and ZAMIRI (2009). In the current study, all additives significantly ($P \leq 0.01$) decreased the percentage of ammonia nitrogen in total nitrogen, which is an indicator of the degradation of nitrogen compounds in silage (GUO et al. 2008, 2011). Other authors (GUO et al. 2008, FIJAŁKOWSKA et al. 2015) noted similar effects of fermentation inhibitors and/or stimulants that reduced the percentage of ammonia nitrogen by impeding deamination and bacterial decarboxylation. High concentrations of ammonia nitrogen (>12% to 15% of total nitrogen) result from excessive protein breakdown caused by a slow drop in pH or the activity of *Clostridium* (PURWIN et al. 2009), which was not observed in the current study, pointing to effective fermentation in silages with additives. According to PURWIN et al. (2009), silages with lower DM content have higher concentrations of ammonia nitrogen, which was noted in the control silage in our experiment.

An analysis of the apparent digestibility in adult rams revealed high digestibility coefficients of all analyzed nutrients in *Sida* silages. The coefficients of CP digestibility were higher than those reported for grass and legume silages (SOBIECH et al. 2015). The tested additives had a significant effect only on the digestibility of NDF and CP (Table 3). The bacterial inoculant and organic acids significantly increased CP digestibility ($P \leq 0.01$ and $P \leq 0.05$, respectively) and decreased NDF digestibility ($P \leq 0.05$ and $P \leq 0.01$, respectively). The coefficient of CP digestibility was significantly ($P \leq 0.01$) lower in the variant with grass. A significant increase in NDF digestibility was noted in the variants with straw ($P \leq 0.05$), grass and sugar beet pulp ($P \leq 0.01$) – Table 3. The analyzed additives had no influence on the digestibility of DM or OM. SPEIJERS et al. (2005) demonstrated that grass and alfalfa silages had different concentrations of NDF and ADF, and alfalfa silage had a higher content of CP and ammonia nitrogen. In the cited study, alfalfa silage was more willingly consumed by lambs, and silage intake was strongly correlated with the cell wall content and digestibility. In the current experiment, Virginia fanpetals ensiled with a blend of organic acids was

Table 3

Digestibility coefficients (%) of nutrients and the digestible organic matter content of Virginia fanpetals silage

| Silage | DM | OM | CP | NDF | dOM (g kg$^{-1}$ DM) | NEL (MJ kg$^{-1}$) |
|---|---|---|---|---|---|---|
| CO | 70.7 | 72.4 | 92.1 | 60.9 | 662 | 5.63 |
| SS | 73.8 | 75.5 | 91.2 | 62.5$^{ab}$ | 694 | 5.75 |
| SG | 74.5 | 77.0 | 89.3$^{Bb}$ | 67.4$^{Aab}$ | 698 | 5.30 |
| SM | 75.2 | 77.0 | 91.2 | 56.8 | 712 | 6.00 |
| SB | 74.3 | 76.0 | 90.2 | 67.2$^{Aac}$ | 711 | 6.48 |
| SI | 74.1 | 74.2 | 93.8$^{A}$ | 56.0$^{bd}$ | 679 | 5.91 |
| SA | 71.4 | 72.7 | 92.8$^{a}$ | 50.5$^{Bcd}$ | 668 | 6.00 |
| SEM | 0.83 | 0.84 | 0.43 | 1.56 | 0.79 | 0.14 |

DM – dry matter, OM – organic matter, CP – crude protein, NDF – neutral detergent fiber, dOM – digestible organic matter, NEL - net lactation energy, SEM – standard error of the mean, significance levels: *a*, *b* – $P \leq 0.05$, *A*, *B* – $P \leq 0.01$.

Silage types: CO – control, without additives, SS – with the addition of straw (2 kg), SG – with the addition of grass (1:1), SM – with the addition of molasses (30 g kg$^{-1}$ FM), SB – with the addition of dried sugar beet pulp pellets (9:1), SI – with the addition of a bacterial inoculant: *Lactobacillus plantarum, Lactobacillus buchneri, Lactococcus lactis* ($2.5 \cdot 10^8$ CFU kg$^{-1}$ FM), SA – with the addition of a blend of organic acids.

characterized by the lowest NDF digestibility, which was significantly ($P \leq 0.05$) lower relative to silages with the addition of grass, sugar beet pulp and straw. In contrast, Sobiech et al. (2015) found that ADF digestibility was the highest in alfalfa silage, whereas NDF digestibility was relatively low. Sobiech et al. (2015) also noted higher DM intake in lambs fed red clover silage than in those receiving grass silage. Both red clover silage and alfalfa silage had higher intake potential than grass silage at the same digestibility levels. In a study by Andrae et al. (2001), commonly fed corn silage was characterized by lower digestibility of DM (55%) and NDF (36%). The energy value of silages was at a similar level, except for the SG variant, with the lowest NEL. SB silage was characterized by the highest energy concentration.

# CONCLUSIONS

The quality of Virginia fanpetals silages produced with the use of various additives was evaluated as high or very high. The tested additives significantly reduced the utilization of WSC, decreased the fraction of structural sugars and positively affected the fermentation profile of silages by decreasing the concentrations of acetic acid and butyric acid as well as ammonia nitrogen, which is an important indicator of silage quality. In all *Sida* silages, the digestibility of OM and NDF was similar to the values determined in alfalfa silage made from herbage harvested in the bud formation stage. Butyric acid concentration was the key factor deteriorating the quality of *Sida* silage. The content of butyric acid was lowered only in silages containing sugar beet pulp and grass. Molasses and sugar beet pulp had the most beneficial influence on the content of digestible OM in silage. All analyzed additives limited proteolysis during ensiling. Dried sugar beet pulp was the most effective additive, which positively affected the chemical composition, fermentation profile and apparent digestibility of *Sida* silage.

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