



## Effect of *Lactobacillus buchneri* on the nutritive value of Sucrosorgo 506 bagasse silage

M. Wilk<sup>1,4</sup>, B. Król<sup>1</sup>, M. Słupczyńska<sup>1</sup>, J. Sowiński<sup>2</sup> and Z. Antoszkiewicz<sup>3</sup>

Wrocław University of Environmental and Life Sciences, <sup>1</sup> Faculty of Biology and Animal Science, Department of Animal Nutrition and Feed Science, Chelmońskiego 38C, 51-630 Wrocław, Poland

<sup>2</sup> Faculty of Life Sciences and Technology, Institute of Soil Science and Environmental Protection, Grunwaldzka 53, 50-357 Wrocław, Poland

<sup>3</sup> University of Warmia and Mazury, Faculty of Bioengineering, Department of Animal Nutrition and Feed Science, Oczapowskiego 5, 10-719 Olsztyn, Poland

**KEY WORDS:** sorghum bagasse, Sucrosorgo 506, silage, *Lactobacillus buchneri*, nutritive value, *in vitro* degradability

Received: 25 January 2020  
Revised: 18 May 2020  
Accepted: 10 June 2020

<sup>4</sup> Corresponding author:  
e-mail: [martyna.wilk@upwr.edu.pl](mailto:martyna.wilk@upwr.edu.pl)

**ABSTRACT.** Sweet sorghum bagasse (SSB) is a dry pulpy residue that remains after the extraction of juice from *Sorghum saccharatum* for ethanol production, and represents unused plant biomass that can be used in livestock feeding. The aim of the present study was to determine the effect of heterofermentative bacteria *Lactobacillus buchneri*, added during ensiling process to Sucrosorgo 506 bagasse, on anaerobic stability, nutritional value, *in vitro* ruminal and intestinal disappearances and quality of silage in micro-silos. In this experiment, the inoculant was applied at a rate of  $5 \times 10^4$  CFU/ml (B1). Sucrosorgo 506 bagasse silage without the additive served as control (B0). The basic composition, the content of minerals and the amino acid profile were determined for both fresh and ensiled materials. The aerobic stability,  $\text{NH}_3\text{-N}$  content and pH value of the silage were determined to evaluate its quality. A positive effect of the addition of *L. buchneri* on the nutritional value and quality, especially volatile fatty acid profile was revealed. It was also found that both investigated Sucrosorgo 506 bagasse silages were aerobically stable and exhibited satisfactory quality. The silage prepared with *L. buchneri* had better effective ruminal degradability and intestinal digestibility *in vitro*.

### Introduction

Sorghum is grown in African countries, the USA, Mexico, India and China, and represents one of the most important crops in the world. The beneficial qualities of sorghum include high biomass yield, economical water management, well-developed root system, which allows absorption of water even from layers present at a depth of 1.5 m underground during drought conditions, and ability to grow under deprived soil conditions where other cereal crops cannot grow (Promkhambut

et al., 2010). The changes observed in the European climatic conditions have forced local farmers to look for plants that will be able to thrive during extreme conditions and produce high-yield crops with excellent biomass quality. Owing to the beneficial qualities of sorghum and the fact that it provides a potential renewable biomass resource, it is also grown as a crop in European countries as it produces the highest concentration of carbohydrates and sugars per hectare. It has the potential to become a significant supplier of biomass for feed, fuel and fibre (Eggleston et al., 2013). The majority

of sorghum biomass is constituted by juice which contains sugars such as sucrose, glucose and fructose. The sugar-rich juice of sweet sorghum is used for the production of bioproducts, ethanol and biofuels (Ratnavathi et al., 2016). Sugar extraction from the stalks provides a solid, cellulosic residue (bagasse) as the by-product which accounts for about 30% of the fresh plant biomass. The major portion of bagasse remains underutilized, and only a small percentage is used in the fields to enhance the subsequent yield. Hence, the use of bagasse in other areas is being explored. Due to higher protein content as compared to sugarcane, sweet sorghum bagasse (SSB) is considered to be more valuable as an animal feed (Venkata Seshaiyah et al., 2012; Egglestone et al., 2013; Naeini et al., 2014). However, this plant shows low stability, and hence its biomass needs to be conserved. One of the most popular and effective methods of forage preservation is ensiling. The standard practice that has been followed to improve the fermentation efficiency is by supplementing with various silage additives (McDonald et al., 2011), such as *Lactobacillus acidophilus*, *L. plantarum*, *Enterococcus faecium*. These bacteria ferment water-soluble carbohydrates (WSC) into organic acids, which reduce the pH of the surrounding environment and thus inhibit the activity of aerobic bacteria. Moreover, lactic acid bacteria (LAB) accelerate lactic acid fermentation and reduce nutrition losses during the ensilage process (Wang et al., 2019). As reported by Fijałkowska et al. (2020), the addition of LAB and combination of similar strains, to silage ‘completely or almost completely inhibited the growth of toxin-producing fungi of the genera *Aspergillus* and *Fusarium*’. In order to increase the stability of silages against contamination by yeasts and molds, in 1996 scientists proposed the use of *L. buchneri* as a silage additive. Garzia and Giovanna (1984), over 35 years ago, showed that among the 40 strains of *Lactobacillus* identified (in 23 silage samples), 33 were heterofermentative in nature. This finding suggests that in naturally fermented silage, various heterofermentative strains contribute to acid production, at least in the final stages of fermentation. The most frequently found heterofermentative LAB was *L. buchneri*. This heterofermentative bacteria leads to secondary oxidation of lactic acid to acetic acid, and thereby inhibits the proliferation of some yeast species that are responsible for heat production under aerobic conditions, thus increasing the stability of air-exposed silage. Supplementation with additives can improve the quality of silage and result in increased intake among animals, digestibility of

dry matter (DM), and hence the animal health and performance (Kostulak-Zielińska and Potkański, 2001; McDonald et al., 2011).

So, the aim of the present study was to determine the impact of *L. buchneri* ( $5 \times 10^4$  CFU/ml), added to the ensiled Suscrosorgo 506 bagasse, on nutritional value, quality, *in vitro* ruminal degradability and intestinal digestibility, and anaerobic stability of the obtained silage products.

## Material and methods

### Research material

Feedstock for silage of sweet sorghum (*Sorghum saccharatum*) was obtained from Sucrosorgo 506 hybrid. Field experiment was carried out at Wrocław University of Environmental and Life Sciences at the station located of southwestern Poland (N 51°10'25" E 17°07'02"). Sucrosorgo 506 (Sorghum Partners Inc., Longmont, CO, USA) is a late-maturing sweet sorghum hybrid well adapted in the moderate climate of Central Europe. The Sucrosorgo 506 hybrids were sown using a Wintersteiger plot drill at the beginning of May, at sowing rate 20 germinated seeds per m<sup>2</sup>, at sowing depth of 3–5 cm. The annual N, P, K input was 100, 70 and 100 kg/ha respectively of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O. Fertilizer was broadcast before sowing. The sorghum plants were hand harvested using a brush cutter at the end of September and chopped with bowl chopper (Krag, Poland) with chop length  $6 \pm 2$  mm. Shredded biomass of Sucrosorgo 506 subsample was obtained for juice extraction (piston press, 30 bar pressure, Hydropras Skalar, Warsaw, Poland). Dry matter yield of Sucrosorgo biomass was  $11.75 \pm 4.13$  ton DM per ha (Głąb et al., 2019).

During the experiment, Sucrosorgo 506 bagasse was ensiled (PVC-tubes, about 2 kg per each), with *L. buchneri* ( $5 \times 10^4$  CFU/ml) (B1), and the bagasse sample with no additive served as control (B0). *L. buchneri* was mixed with water and sprayed to the silage batches prior to filling. To ensure precise mixing of bacterial inoculant to the plant biomass, first a small portion of the material was mixed with *L. buchneri* and then the portion was mixed with whole batch thoroughly. After filling the silos, plant material was pressed to ensure expelling of the air, the silos were closed tightly. Each treatment was performed in six replicates and the samples were ensiled in the silage laboratory of the Department of Animal Nutrition and Feed Science, Wrocław University of Environmental and Life Sciences for 180 days at a temperature of about 19 °C.

### Proximate analysis

In fresh bagasse and obtained silage samples, proximate analysis was performed for the following variables: dry matter (DM, weight method, AOAC: 934.01); true protein (TP), as difference between the crude protein (CP) and the soluble non protein-nitrogen fraction (NPN) with the use of a FOSS Tecator 2300 Kjeldtec Analyzer Unit (FOSS Tecator, Hoganas, AB, Sweden) (Kjeldahl method, AOAC: 984.13); crude ash (CA, weight method: 942.05); ether extract (EE, Soxhlet method, AOAC: 920.39A) with the use of a BUCHI Extraction System B-811 (BÜCHI, Flawil, Switzerland); crude fibre (CF, Hanneberg and Stohmann method, AOAC: 978.10); fibre fractions such as neutral detergent fibre (NDF) and acid detergent fibre (ADF, AOAC: 973.18) with the use of a Fibertec 2010 apparatus FOSS (FOSS Fibertec, Hoganas, AB, Sweden) (AOAC, 2011). The nitrogen-free extractives (NFE) and the non-fibre carbohydrates (NFC) were calculated in g/kg of DM using the formulas:

$$NFE = 1000 - (CP + CA + CF + EE),$$

$$NFC = 1000 - (CA + CP + EE + NDF),$$

where: *NFE* – nitrogen-free extractives, *CP* – crude protein, *CA* – crude ash, *CF* – crude fibre, *EE* – ether extract, *NFC* – non-fibre carbohydrates, *NDF* – neutral detergent fibre.

Additionally the concentration of water-soluble carbohydrates (WSC) was determined using the anthron method (Yemm and Willis, 1954).

The mineral content of the investigated bagasse samples was determined by spectrophotometric method which involved the use of a VARIAN AA240FS atomic absorption spectrometer (AA240FS, Varian, Palo Alto, CA, USA). The readings were taken at wavelength of 422.7 nm for detecting calcium (Ca), at 285.2 nm for magnesium (Mg), at 589.0 nm for sodium (Na), and at 766.5 nm for potassium (K; AOAC: 975.03, AOAC, 2011). The phosphorus (P) content was determined at wavelength of 470.0 nm using the spectrophotometric method with the use of a PC SPECTRO Aqua Lytic (Aqualytic, Dortmund, German) (AOAC: 985.01, AOAC, 2011).

The amino acid (AA) profile was determined with the use of an Amino Acids Analyzer AAA 400 (INGOS, Prague, the Czech Republic) according to standard protocol (AOAC: 994.12, AOAC, 2011). Tryptophan was determined using a spectrophotometer 2000 RS (Aqualytic, Dortmund, German) at a wavelength of 590.0 nm (AOAC: 988.15, AOAC, 2011). Volatile fatty acids (VFA) and ethanol were determined by high-performance liquid chromatography (HPLC) using a Shimadzu HPLC apparatus (Shimadzu,

Japan) (Kostulak-Zielińska and Potkański, 2001). The content of  $\text{NH}_3\text{-N}$  (Kjeldahl method, AOAC: 941.04) in the tested samples was determined (AOAC, 2011). In addition, the buffer capacity (BC) of the fresh bagasse and obtained silages were determined (Moharrery, 2007). The aerobic stability of silages was also evaluated in the bulk samples. The aerobic stability was calculated as the number of hours that the silage temperature remained stable before rising more than 2 °C above the baseline temperature. The temperature was recorded for 5 days (125 h) using an automatic, electronic, multichannel thermometer LB-711 (LAB-EL, Reguły, Poland) at an average temperature of 21 °C.

### *In vitro* rumen degradability and intestinal digestibility

Three Polish Holstein Friesian dry cows of average body weight 600 kg fitted with rumen canula were used as rumen fluid donors. The animals were kept in individual pens with constant access to water and mineral blocks. The animals were fed maintenance diet consisting of 70% forage (hay) and 30% concentrate on DM basis. The feeds were given to animals twice a day (8:00 and 16:00). The rumen fluid was collected 2 h after morning feeding and immediately taken to the laboratory in thermos flasks. The rumen fluid was filtered by 4-layer cheesecloth and kept in anaerobic conditions by flushing  $\text{CO}_2$ . The feed samples were ground to pass 2 mm sieve and used for *in vitro* degradability method (Daisy II Incubator, ANKOM Technology, Macedon, NY, USA).

The feed samples were incubated in a 6-fold replicate in the rumen fluid for 96, 72, 48, 24, 16, 8, 4, 2 and 0 h. To determine the intestinal disappearance of nutrients samples were incubated in rumen fluid for 16 h as described in effective ruminal degradability (ERD) estimation procedure. After incubation in rumen fluid samples were weighted, put into nylon bags (ANKOM R510, 50  $\mu\text{m}$ ), sealed thermally and placed in Daisy II incubation chamber (ANKOM Technology, Macedon, NY, USA) in a 0.1 N HCl acid solution (pH 1.9) containing pepsin (1 g/l, Cat. No. P-7000, Sigma-Aldrich, St. Louis, MO, USA) Sigma, and incubated with constant rotation for 1 h at 39 °C to imitate digestion in the abomasum. Then the samples in bags were inserted into the incubation chamber with 2 l of pancreatin solution (0.5 M  $\text{KH}_2\text{PO}_4$  buffer, pH 7.75 containing 50 ppm thymol and 3 g pancreatin/l, Cat. no. P-7545, Sigma-Aldrich, St. Louis, MO, USA) and incubated for 24 h with constant rotation at 39 °C to imitate intestinal digestibility. The digestibility was expressed as the amount of nutrient in the rumen fermenta-

tion residue minus nutrient remaining after pepsin-pancreatin incubation divided by the amount of nutrient in the rumen residue (Calsamiglia and Stern, 1995). The effective rumen degradability of DM was calculated in accordance with Ørskov and McDonald (1979):

$$y = a + b \times (1 - e^{-ct})$$

$$ERD = a + \frac{(b \times c)}{(c + k)}$$

where:  $y$  – degradability of analysed component, after incubation time  $t$ ,  $a$  – soluble fraction (%),  $b$  – potential degradability of insoluble fraction (%),  $a + b$  – potential degradability of fraction;  $c$  – degradation rate (%/h), ERD – effective ruminal degradability,  $k$  – passage rate of feed.

### Statistical tests

All the obtained data were analysed using the Shapiro–Wilk test. The results were evaluated statistically by performing a one-way analysis of variance and computed using a statistical package Statistica ver. 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). In the tables arithmetic mean and standard deviation (SD) of obtained data are presented. Significant differences between the means of the groups were verified using the Duncan's multiple range test. Treatment differences with  $P \leq 0.05$  were considered significant and with  $P \leq 0.01$  as highly significant.

## Results

The proximate analysis of basic nutrients and the mineral composition of Sucrosorgo 506 bagasse, and the AA profile are shown in Table 1. The high level of DM, CF and fibre fractions with a lower level of CA, EE, CP, and thus individual AA, and mineral components is a result of the leakage of soluble nutrients during sugar-juice extraction. The proximate analysis of basic nutrients, mineral composition and AA profile of Sucrosorgo 506 bagasse silages are shown in Table 2, Table 3,

**Table 1.** Basic nutrients, mineral composition and amino acids profile of Sucrosorgo 506 bagasse (mean  $\pm$  SD), g/kg DM

Indices		Amount	
Nutrients	DM, g/kg	429.10 $\pm$ 1.31	
	CA	31.78 $\pm$ 0.84	
	CP	67.57 $\pm$ 2.84	
	NPN	15.50 $\pm$ 0.89	
	TP	52.03 $\pm$ 3.16	
	CF	298.40 $\pm$ 17.20	
	NDF	571.67 $\pm$ 19.63	
	ADF	376.93 $\pm$ 23.55	
	EE	37.15 $\pm$ 0.23	
	NFE	565.11 $\pm$ 17.08	
	NFC	291.84 $\pm$ 19.25	
	WSC	99.06 $\pm$ 5.82	
	BC, meq/l	1.38 $\pm$ 0.16	
Minerals	Ca	1.06 $\pm$ 0.07	
	P	1.24 $\pm$ 0.11	
	Na	0.21 $\pm$ 0.04	
	K	1.31 $\pm$ 0.13	
	Mg	0.48 $\pm$ 0.05	
Amino acids	Amount	Amino acids	Amount
	Asp	2.38 $\pm$ 0.02	Leu
Cys	0.22 $\pm$ 0.00	Val	1.26 $\pm$ 0.06
Tyr	0.53 $\pm$ 0.01	Phe	1.21 $\pm$ 0.03
Ser	1.17 $\pm$ 0.01	His	0.55 $\pm$ 0.02
Glu	3.52 $\pm$ 0.07	Lys	1.00 $\pm$ 0.02
Pro	1.42 $\pm$ 0.03	Arg	0.99 $\pm$ 0.00
Gly	1.19 $\pm$ 0.01	Thr	1.13 $\pm$ 0.05
Ala	1.80 $\pm$ 0.03	Met	0.37 $\pm$ 0.01
Ile	0.88 $\pm$ 0.01	Try	0.38 $\pm$ 0.04

and Figure 1, respectively. The influence of the microbial additive on the contents of TP ( $P = 0.048$ ), CA ( $P = 0.025$ ), concentration of NFE ( $P = 0.032$ ) and NFC ( $P = 0.001$ ) is shown. The fibre detergent fractions (NDF ( $P = 0.002$ ) and ADF ( $P = 0.000$ )), CF ( $P = 0.000$ ), WSC ( $P = 0.000$ ) and EE ( $P = 0.002$ ) were significantly lower in B1 sample in comparison to B0 sample. The addition of *L. buchneri* did not affect the DM, CP, NPN, and TD contents (Table 2). The addition of *L. buchneri* showed an

**Table 2.** Chemical composition of Sucrosorgo 506 bagasse silages, g/kg of DM (mean  $\pm$  SD)

Group	DM, g/kg	CA	CP	NPN	TP	CF	NDF	ADF	EE	NFE	NFC	WSC	TD, %	BC, meq/l
B0	389.0 $\pm$ 5.52	33.8 <sup>b</sup> $\pm$ 0.66	70.9 $\pm$ 6.81	12.6 $\pm$ 1.00	58.3 <sup>b</sup> $\pm$ 5.25	362.3 <sup>A</sup> $\pm$ 1.59	707.5 <sup>A</sup> $\pm$ 6.76	436.3 <sup>A</sup> $\pm$ 3.28	46.8 <sup>A</sup> $\pm$ 0.06	486.2 <sup>b</sup> $\pm$ 9.68	141.0 <sup>B</sup> $\pm$ 1.33	31.7 <sup>A</sup> $\pm$ 0.61	34.67 $\pm$ 4.34	2.78 $\pm$ 0.34
B1	381.5 $\pm$ 4.74	35.0 <sup>a</sup> $\pm$ 0.49	77.9 $\pm$ 0.60	11.3 $\pm$ 0.59	66.9 <sup>a</sup> $\pm$ 0.20	343.5 <sup>B</sup> $\pm$ 1.48	682.0 <sup>B</sup> $\pm$ 4.58	410.9 <sup>B</sup> $\pm$ 2.13	44.6 <sup>B</sup> $\pm$ 0.77	499.0 <sup>a</sup> $\pm$ 0.61	160.5 <sup>A</sup> $\pm$ 5.45	22.6 <sup>B</sup> $\pm$ 0.51	34.37 $\pm$ 2.48	2.38 $\pm$ 0.20
<i>P</i> -value	0.064	0.025	0.125	0.111	0.048	0.000	0.002	0.000	0.002	0.032	0.001	0.000	0.923	0.158

<sup>ab, AB</sup> – values in columns with different superscripts are significantly different at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

**Table 3.** Amino acids profile of Sucrosorgo 506 bagasse silages, g/kg of DM (mean  $\pm$  SD)

Amino acids, g/kg DM	Group		P-value
	B0	B1	
Asp	3.64 <sup>A</sup> $\pm$ 0.22	3.11 <sup>B</sup> $\pm$ 0.12	0.006
Cys	0.60 $\pm$ 0.02	0.58 $\pm$ 0.04	0.313
Tyr	0.89 $\pm$ 0.04	0.81 $\pm$ 0.01	0.597
Ser	1.48 <sup>b</sup> $\pm$ 0.15	1.91 <sup>a</sup> $\pm$ 0.02	0.033
Glu	4.33 $\pm$ 0.32	4.44 $\pm$ 0.15	0.269
Pro	2.21 $\pm$ 0.19	2.56 $\pm$ 0.34	0.098
Gly	1.75 <sup>A</sup> $\pm$ 0.18	1.14 <sup>B</sup> $\pm$ 0.12	0.007
Ala	1.66 <sup>a</sup> $\pm$ 0.04	1.42 <sup>b</sup> $\pm$ 0.04	0.038
Ile	1.87 <sup>A</sup> $\pm$ 0.11	1.21 <sup>B</sup> $\pm$ 0.25	0.004
Leu	2.67 $\pm$ 0.29	2.56 $\pm$ 0.36	0.592
Val	1.15 <sup>b</sup> $\pm$ 0.17	1.44 <sup>a</sup> $\pm$ 0.17	0.042
Phe	1.60 <sup>a</sup> $\pm$ 0.02	1.24 <sup>b</sup> $\pm$ 0.24	0.021
His	0.84 <sup>a</sup> $\pm$ 0.06	0.55 <sup>b</sup> $\pm$ 0.05	0.023
Lys	1.30 $\pm$ 0.11	1.40 $\pm$ 0.10	0.156
Arg	1.09 $\pm$ 0.00	1.37 $\pm$ 0.00	0.156
Thr	1.43 $\pm$ 0.27	1.76 $\pm$ 0.14	0.058
Met	0.92 $\pm$ 0.01	0.94 $\pm$ 0.04	0.335
Try	0.30 $\pm$ 0.01	0.31 $\pm$ 0.01	0.101

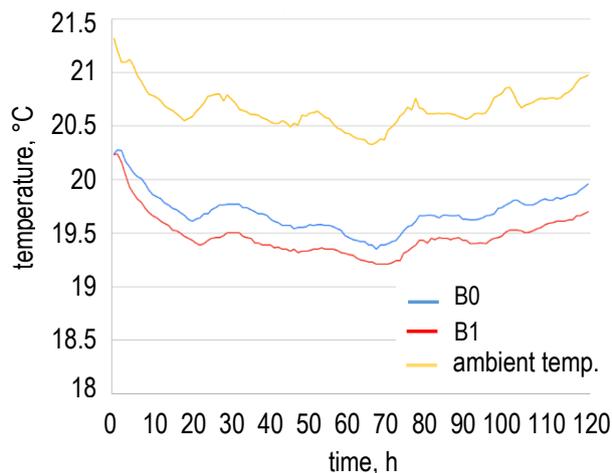
<sup>ab, AB</sup> – values in rows with different superscripts are significantly different at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

impact on contents of Asp, Gly, Ile ( $P \leq 0.01$ ) and Ser, Ala, Val, Phe, His ( $P \leq 0.05$ ) (Table 3). The influence of *L. buchneri* on the content of total VFAs ( $P = 0.001$ ), the content of all analysed VFAs (excluding valeric acid and isovaleric acid), and pH index ( $P = 0.010$ ) was found. No differences were stated in buffering capacity between the analysed samples (Table 4). All the analysed silage samples showed aerobic stability, during the monitoring period for 125 h (Figure 1).

**Table 4.** Fermentation profile of Sucrosorgo 506 bagasse silages (mean  $\pm$  SD)

Indices	Group		P-value
	B0	B1	
Total VFA include, g/kg DM:	114.96 <sup>A</sup> $\pm$ 12.71	225.16 <sup>B</sup> $\pm$ 17.54	0.000
lactic	86.15 <sup>A</sup> $\pm$ 13.68	180.30 <sup>B</sup> $\pm$ 16.04	0.000
acetic	5.91 <sup>A</sup> $\pm$ 1.17	33.33 <sup>B</sup> $\pm$ 1.98	0.000
butyric	19.44 <sup>A</sup> $\pm$ 0.75	9.79 <sup>B</sup> $\pm$ 0.50	0.000
isobutyric	3.41 <sup>A</sup> $\pm$ 0.57	1.71 <sup>B</sup> $\pm$ 0.02	0.002
formic	0.05 <sup>A</sup> $\pm$ 0.01	0.00 <sup>B</sup> $\pm$ 0.00	0.000
propionic	0.00 <sup>A</sup> $\pm$ 0.00	0.02 <sup>B</sup> $\pm$ 0.00	0.000
isovaleric	0.00 $\pm$ 0.01	0.01 $\pm$ 0.01	0.678
valeric	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.378
Ethanol, g/kg DM	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.374
NH <sub>3</sub> -N, %N <sub>total</sub>	3.41 $\pm$ 1.00	3.92 $\pm$ 0.54	0.304
pH	4.01 <sup>A</sup> $\pm$ 0.04	3.91 <sup>B</sup> $\pm$ 0.04	0.010

<sup>ab, AB</sup> – values in rows with different superscripts are significantly different at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

**Figure 1.** Aerobic stability of B0 and B1 silages

The sorghum bagasse treated with *L. buchneri* was characterised by a higher rapidly degradable fraction ( $P = 0.039$ ), potentially degradable fraction ( $P = 0.049$ ) and organic matter digestibility ( $P = 0.046$ ) than silages without this additive. A tendency to higher effective rumen degradability of inoculant in the treated silage was also observed (Table 5).

**Table 5.** *In vitro* rumen degradability and intestinal digestibility

Indices	Group		P-value
	B0	B1	
Rapidly degradable fraction (a), g/kg	178 <sup>b</sup>	198 <sup>a</sup>	0.039
Slowly degradable fraction (b), g/kg	567	563	0.122
Degradation rate (kd), %/h	3.17	3.26	0.565
Potentially degradable fraction (a+b), g/kg	746 <sup>b</sup>	772 <sup>a</sup>	0.049
ERD with a rate of passage of 4%/h	568	589	0.069
ERD with a rate of passage of 6%/h	530	549	0.072
Organic matter digestibility (OMD), %	42.35 <sup>b</sup>	44.31 <sup>a</sup>	0.046

<sup>ab, AB</sup> – values in rows with different superscripts are significantly different at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

## Discussion

The divergence in SSB chemical composition between our results and the data reported by other authors (Naeini et al., 2014) may result from differences in the sorghum variety, soil fertilization techniques, dates of harvesting, climatic conditions, and juice extraction process. As reported by Muck and Shinnors (2001), the DM concentration should range between 35 and 45% for optimum silage conditions, the DM concentration between 27–38% provides proper compactness to the plant material, promotes rapid fermentation and minimizes DM losses during the ensilage process being a consequence of the leakage of silage effluent.

The DM content of Sucrosorgo 506 bagasse used in the experiment was 42.91%. Low concentration of WSC in fresh bagasse (similar to value obtained by Naeini et al., 2014) is a consequence of the juice extraction and it was expected. The juice extracted from sweet sorghum stems contained high sugar contents and could be directly fermented by microbes (Liu and Shen, 2008). The sufficient WSC contents of SSB and the contents of NDF and ADF, which were lower in comparison to the results reported by Bernardes et al. (2016), indicated that this forage could achieve proper fermentation profile during ensiling (Naeini et al., 2014). The CP and TP contents were similar to values obtained by other authors (Houx et al., 2013). As reported by Naeini et al. (2014) the concentration of CP at 55–90 g/kg of DM may result in longer time for fermentation. Due to the fact that sorghum cultivated in Polish climate conditions does not produce mature seeds, and the juice extraction by high pressure leads to protein denaturation (Houška and da Silva, 2017) the AA values obtained in this study were lower than in other previous studies (Tedeschi et al., 2001).

The WSC as the main energy source for microorganisms enhance forage fermentation and thus raising the silage quality. Compared to the fresh bagasse the concentration of WSC decreased in both obtained silages (greater decrease in B1 silage), due to the microbial activity of applied inoculant that produce lactic acid (higher in B1) to drop the silage pH.

The pH values between 3.8 and 4.2 testifies well-preserved silage (McDonald et al., 2011), and the pH values of both the investigated silage samples were within this range. The lower pH value was statistically confirmed ( $P = 0.010$ ), which may be related to higher NFE ( $P = 0.032$ ) and NFC ( $P = 0.001$ ) concentrations. Generally, the protein analysis determines the CP (based on total nitrogen) and the content of ammonia in biomass. Ammonia and amines that neutralize acids are the product of deamination and decarboxylation of AA. During the fermentation process, the breakdown of proteins continues, but this activity decreases as the pH falls. LAB, as an additive, has been shown to accelerate the acidification process, inhibit the compounds involved in proteolysis and increase the aerobic stability (McDonald et al., 2011), what found confirmation in higher content of TP ( $P = 0.048$ ) in bagasse silage treated with *L. buchneri*. In our own studies, *L. buchneri* did not affect the CP value ( $P = 0.125$ ) and NPN value ( $P = 0.111$ ) but may act on the nitrogenous compounds produced as a result of proteolysis, such as AAs and peptides. Statistically significant differences in the AA

content in treatments were found. Proteins present in the silage are highly degradable in the rumen, and free AAs are rapidly metabolized. However, AAs fed to ruminants in an unprotected form were characterized by high rumen escape values; therefore, AA composition of silage may be nutritionally significant (McDonald et al., 2011). The obtained data show that all silage products had a similar composition, but the addition of bacterial inoculant (*L. buchneri*) could affect the breakdown of peptide bonds and thus the levels of the analysed AA. The contents of Asp, Gly and Ile ( $P \leq 0.01$ ) were lower in B1 when in comparison to B0 group. *Escherichia coli*, which grows at the beginning of the ensiling process, is known to deaminate Asp and Glu. Similar activity has also been observed with heterofermentative *L. brevis*, which may explain the decrease in the concentration of these AA in silage with the addition of *L. buchneri* (Heron et al., 1993). In contrast, the levels of Ser and Val ( $P \leq 0.05$ ) were higher in B1 in comparison to B0 group. In well-preserved silages, deamination of AA may occur at a low rate during fermentation. As confirmed by McDonald et al. (2011), silage with  $\text{NH}_3\text{-N}$  concentration below 7% of the total N is considered excellent and between 7–10% is considered good. The  $\text{NH}_3\text{-N}$  values determined in this study were 3.41% for B0 and 3.92% for B1 samples, which revealed no statistically significant differences. Low levels of  $\text{NH}_3\text{-N}$  can be correlated with low concentration of NPN, which is rapidly converted to ammonia in the rumen. The  $\text{NH}_3\text{-N}$  shows the degree of protein degradation and increased concentration of  $\text{NH}_3\text{-N}$  could be a result of increased degradation by bacterial activities. The  $\text{NH}_3\text{-N}$  concentration can arise from the reduction of nitrates, nitrites, and the effect of microbial activity (AA fermentation).

In addition, the pH values of both bagasse silages were well below the maximum pH value (4.8) recommended for silage with concentration of DM at 350–400 g/kg of fresh matter in order to avoid the development of butyric acid bacteria. The decrease in pH values by *L. buchneri* inoculant in B1 silage was statistically significant ( $P = 0.010$ ). The final silage pH depends on many factors, especially the concentration of lactic acid and buffering capacity. Buffering capacity determines to what degree a forage will resist a change in pH. The buffer capacity of the obtained sorghum bagasse silages is lower compared to the silages analysed by Moharrery (2007), however it is higher than the fresh sugar cane bagasse.

Due to the presence of high content of sucrose in sorghum bagasse, there is a risk of sugar getting converted into alcohol, which consequently reduces the nutritional value of the product. Hence, safety measures need to be taken to ensure that only LAB grow but not yeast. The higher concentration of lactic acid, acetic acid and propionic acid in silage with *L. buchneri* inoculation were expected, due to the heterolactic fermentation profile where sugars are fermented to several end-products, mainly lactic acid, and also large amounts of acetic acid and 1,2-propanediol, which may be converted to propionic acid (Oude et al., 2001). The Sucrosorgo 506 bagasse silages were dominated by lactic acid, and in our study lactic acid concentrations were found to be higher than the minimum value (30 g/kg of DM) for proper lactic fermentation. The statistically significant ( $P = 0.000$ ) higher content of acetic acid in silage inoculated with *L. buchneri* is indicative of heterofermentation (Fijałkowska et al., 2020).

Most importantly, the presence of *Clostridia* is considered undesirable during the ensiling process because it can produce butyric acid from WSC and proteins. According to Ward et al. (2001) other acids i.e., isobutyric and isovaleric acids could also result from secondary clostridial fermentation of lactic acid. McDonald et al. (2011) reported that, silage with butyric acid concentration exceeding 10 g/kg of DM should not be fed to animals. Most LAB inoculants lower the level of this acid, and addition of *L. buchneri* resulted in a statistically significant decrease ( $P = 0.000$ ) in the concentration of butyric acid and isobutyric acid ( $P = 0.002$ ) which may be also correlated with significant lower deamination of valine ( $P = 0.042$ ), as well as lower protein degradation (higher content of TP,  $P = 0.048$ ). No effect of the bacterial additive on the concentration of isovaleric acid was demonstrated, which was confirmed by the lack of differences in the concentration of leucine in the analysed silages. Unfortunately, *Clostridia* is often detected in grass silages. It is possible that high DM content could efficiently inhibit the growth of *Clostridium*, especially in B0 silage (Jatkauskas et al., 2013). An increased amount of acetic acid may decrease the palatability of fodder and reduce DM intake. However, acetic acid produced by *L. buchneri* inhibits the proliferation of some yeast species, thus improving the aerobic stability of silage and preventing the metabolization of lactic acid to alcohol (Danner et al., 2003). There were no traces of ethanol in the analysed silage samples. Aerobic stability is a crucial element of silage, which indicates that all the included nutrients have

been well-preserved. Weinberg et al. (2002) reported that while testing for aerobic stability, silages with *L. buchneri* additive were found to be stable, whereas silages treated with *L. plantarum* were deteriorated. The silages obtained in this study were characterized by aerobic stability with good visual appearance, smell and colour. Due to fungicidal properties, *L. buchneri* is added to preserve the quality of silage and protect it from deterioration (Morvay et al., 2011).

Most soluble compounds are extracted during pressing, thereby increasing the fibre concentration and reducing the overall digestibility of bagasse and bagasse silage. The CF of silage samples B0 and B1 constituted 36.23 and 34.36% of DM, respectively. *L. buchneri* addition in ensiling process increased effective rumen degradability (ERD) of sorghum bagasse and organic matter digestibility (IVOMD) *in vitro*. Treatment with microbial inoculation also increased the rapidly and potentially degradable DM. It could be a result of potential of ferulic acid esterase (FAE) produced by *L. buchneri* in improving the fibre digestibility of silages that has been reported (Kang et al., 2009). FAE breaks down the linkage between ferulate and polysaccharide chain, and hydrolyses the linkage between lignin and hemicellulose in the plant cell walls (Yu et al., 2005). Bingöl and Baytok (2003) suggested that the increased concentration of LAB in sorghum silages contributes to NDF and ADF degradation and thus to decreased fibre fraction content in the final product. This finding is confirmed by the results obtained in this study. This indicates that it can effectively increase the bioavailability of undegradable carbohydrate fractions of sorghum bagasse silage with high fibre content (Bean et al., 2009). The microbial activity and acidic environment during ensiling may also lead to increase in degradability of cell walls components. The similar tendency was also stated by Thomas et al. (2013) who also investigated the nutritional value and ruminal kinetics of silages of various sorghum cultivars treated with various inoculants. However, Williams and Shinnors (2012) demonstrated that treatment of sorghum forage harvested at hard dough stage in ensiling process with bacterial inoculant did not improve the degradability of cell wall constituents.

## Conclusions

The chemical composition of fresh Sucrosorgo 506 bagasse was sufficient to ensure adequate fermentation, as indicated by low pH, low  $\text{NH}_3\text{-N}$  concentrations and high lactate concentrations in the bagasse

silages. Good-quality silage can be produced from sweet sorghum bagasse without any additives. However, the addition of *Lactobacillus buchneri* resulted in extensive heterolactic fermentation and thus increased concentrations of lactic acid and acetic acid. The addition of *L. buchneri* had a positive effect on the nutritive value, quality and *in vitro* ruminal degradability and intestinal digestibility of Sucrosorgo 506 bagasse silage.

## References

- AOAC International, 2011. Official Methods of Analysis of AOAC, 18<sup>th</sup> Edition. 4<sup>th</sup> Revision. Gaithersburg, MD (USA)
- Bean B., McCollum T., Villarreal B., Blumenthal J., Robinson J., Brandon R., Buttrey E., Van Meter R., Pietsch D., 2009. Texas panhandle forage sorghum silage trial. AgrLife Research and Extension Center at Amarillo. Accessed May, 2020, <https://amarillo-tamu.edu/wpengine.netdna-cdn.com/files/2010/11/Forage-Sorghum-2009-Variety-Report-Final.pdf>
- Bernardes A.P., Tremblay G.F., Bélanger G., Séguin P., Brégaré A., Vanasse A., 2016. Bagasse silage from sweet pearl millet and sweet sorghum as influenced by harvest dates and delays between biomass chopping and pressing. *Bioenerg. Res.* 9, 88–97, <https://doi.org/10.1007/s12155-015-9666-2>
- Bingöl H.T., Baytok E., 2003. The effects of some silage additives in sorghum silage on the silage quality and ruminal degradability of nutrients. I. The effects on silage quality. *Turk. J. Vet. Anim. Sci.* 27, 15–20
- Calsamiglia S., Stern M.D., 1995. A three-step *in vitro* procedure for estimating intestinal digestion of proteins in ruminants. *J. Anim. Sci.* 73, 1459–1465, <https://doi.org/10.2527/1995.7351459x>
- Danner H., Holzer M., Mayrhuber E., Braun R., 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.* 69, 562–567, <https://doi.org/10.1128/AEM.69.1.562-567.2003>
- Eggleston G., Cole M., Andrzejewski B., 2013. New commercially viable processing technologies for the production of sugar feedstocks from sweet sorghum (*Sorghum bicolor* L. Moench) for biofuel and bioproducts manufacture. *Sugar Tech.* 15, 232–249, <https://doi.org/10.1007/s12355-013-0229-6>
- Fijałkowska M., Przemieniecki S.W., Purwin C., Lipiński K., Kurowski T.P., Karwowska A., 2020. The effect of an additive containing three *Lactobacillus* species on the fermentation pattern and microbiological status of silage. *J. Sci. Food Agric.* 3, 1174–1184, <https://doi.org/10.1002/jsfa.10126>
- Garzia L., Giovanna S., 1984. A survey of lactic acid bacteria in Italian silage. *J. Appl. Bacteriol.* 56, 373–379, <https://doi.org/10.1111/j.1365-2672.1984.tb01364.x>
- Głąb L., Sowiński J., Chmielewska J., Prask H., Fugol M., Szlachta J., 2019. Comparison of the energy efficiency of methane and ethanol production from sweet sorghum (*Sorghum bicolor* L. Moench) with a variety of feedstock management technologies. *Biomass Bioenerg.* 129, 105332, <https://doi.org/10.1016/j.biombioe.2019.105332>
- Heron S.J.E., Wilkinson J.F., Duffus C.M., 1993. Enterobacteria associated with grass and silages. *J. Appl. Bacteriol.* 75, 13–17, <https://doi.org/10.1111/j.1365-2672.1993.tb03401.x>
- Houška M., da Silva V.M.F. (Editors), 2017. High Pressure Processing of Fruit and Vegetable Products. CRC Press. Boca Raton, FL (USA), <https://doi.org/10.1201/9781315121123>
- Houx J.H., Roberts C.A., Fritsch F.B., 2013. Evaluation of sweet sorghum bagasse as an alternative livestock feed. *Crop Sci.* 53, 1784, <https://doi.org/10.2135/cropsci2012.03.0190>
- Jatkauskas J., Vrotniakienė V., Ohlsson Ch., Lund B., 2013. The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. *Agric. Food Sci.* 22, 137–144, <https://doi.org/10.23986/afsci.6698>
- Kang T.W., Adesogan A.T., Kim S.C., Lee S.S., 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. *J. Dairy Sci.* 92, 732–738, <https://doi.org/10.3168/jds.2007-0780>
- Kostulak-Zielińska M., Potkański A., 2001. Quality of baled grass-clover silages ensiled with chemical additives. *Chemical composition. Ann. Anim. Sci.* 1, 153–165
- Liu R., Shen F., 2008. Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized *Saccharomyces cerevisiae* (CICC 1308). *Bioresour. Technol.* 99, 847–854, <https://doi.org/10.1016/j.biortech.2007.01.009>
- McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A., Wilkinson R.G., 2011. *Animal Nutrition*. 7<sup>th</sup> Edition. Trans-Atlantic Publications. Philadelphia, PA (USA)
- Moharrery A., 2007. The determination of buffering capacity of some ruminant's feedstuffs and their cumulative effects on TMR ration. *Am. J. Anim. Vet. Sci.* 2, 72–78, <https://doi.org/10.3844/ajavsp.2007.72.78>
- Morvay Y., Bannink A., France J., Kebreab E., Dijkstra J., 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. *J. Dairy Sci.* 94, 3063–3080, <https://doi.org/10.3168/jds.2010-3995>
- Muck R.E., Shinnors K.J., 2001. Conserved forage (silage and hay): progress and priorities. In: *Proceedings of International Grassland Congress, São Pedro (Brasil)*, 19, 753–762
- Naeni S.Z., Khorvash M., Rowghani E., Bayat A., Nikousefat Z., 2014. Effects of urea and molasses supplementation on chemical composition, protein fractionation and fermentation characteristics of sweet sorghum and bagasse silages as alternative silage crop compared with maize silage in the arid areas. *Res. Opin. Anim. Vet. Sci.* 4, 343–352
- Ørskov E.R., McDonald I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92, 499–503, <https://doi.org/10.1017/S0021859600063048>
- Oude E.S.J., Krooneman J., Gottschal J.C., Spoelstra S.F., Faber F., Driehuis F., 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. *Appl. Environ. Microbiol.* 67, 125–132, <https://doi.org/10.1128/AEM.67.1.125-132.2001>
- Promkhambut A., Younger A., Polthanee A., Akkasaeng C., 2010. Morphological and physiological responses of sorghum (*Sorghum bicolor* L. Moench) to water logging. *Asian J. Plant Sci.* 9, 183–193, <https://doi.org/10.3923/ajps.2010.183.193>
- Ratnavathi C.V., Patil J.V., Chavan U.D., 2016. *Sorghum Biochemistry: An Industrial Perspective*. Academic Press, Elsevier. Cambridge, MA (USA), <https://doi.org/10.1016/B978-0-12-803157-5.00008-3>
- Tedeschi L.O., Pell A.N., Fox D.G., Llamas C.R., 2001. The amino acid profiles of the whole plant and of four plant residues from temperate and tropical forages. *J. Anim. Sci.* 79, 525, <https://doi.org/10.2527/2001.792525x>
- Thomas M.E., Foster J.L., McCuiston K.C., Redmon L.A., Jessup R.W., 2013. Nutritive value, fermentation characteristics, and *in situ* disappearance kinetics of sorghum silage treated with inoculants. *J. Dairy Sci.* 96, 7120–7131, <https://doi.org/10.3168/jds.2013-6635>

- Venkata Seshaiiah C., Ramana Reddy Y., Nagalakshmi D., Jagadeeswara R.S., 2012. Evaluation of sweet sorghum (*Sorghum bicolor* L. Moench) bagasse by chemical, in sacco and in vivo techniques in graded Murrah buffalo bulls. *J. Vet. Adv.* 2, 418–423
- Wang Y., He L., Xing Y., Zheng Y., Zhou W., Pian R., Yang F., Chen X., Zhang Q., 2019. Dynamics of bacterial community and fermentation quality during ensiling of wilted and unwilted *Moringa oleifera* leaf silage with or without lactic acid bacterial inoculants. *mSphere*, <https://doi.org/10.1128/mSphere.00341-19>
- Ward J.D., Redfearn D.D., McCormick M.E., Cuomo G.J., 2001. Chemical composition, ensiling characteristics, and apparent digestibility of summer annual forages in sub-tropical double-cropping system with annual ryegrass. *J. Dairy Sci.* 84, 177–182, [https://doi.org/10.3168/jds.S0022-0302\(01\)74467-0](https://doi.org/10.3168/jds.S0022-0302(01)74467-0)
- Weinberg Z.G., Ashbell G., Hen Y., Azrieli A., Szakacs G., Filya I., 2002. Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*. *J. Ind. Microbiol. Biotechnol.* 28, 7–11, <https://doi.org/10.1038/sj/jim/7000207>
- Williams S.D., Shinnors K.J., 2012. Farm-scale anaerobic storage and aerobic stability of high dry matter sorghum as a biomass feedstock. *Biomass Bioenerg.* 46, 309–316, <https://doi.org/10.1016/j.biombioe.2012.08.010>
- Yemm E.W., Willis A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* 57, 508–514, <https://doi.org/10.1042/bj0570508>
- Yu P., McKinnon J.J., Christensen D.A., 2005. Hydroxycinnamic acids and ferulic acid esterase in relation to biodegradation of complex plant cell walls. *Can. J. Anim. Sci.* 85, 255–267, <https://doi.org/10.4141/A04-010>