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RESEARCH ARTICLE

In vitro Rumen Methanogenesis and Fermentation Profile of Sorghum Whole Crop Cereal and Bagasse Ensilaged with Inoculum *Lactobacillus buchneri*

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ABSTRACT

Lactobacillus buchneri is a heterofermentative bacteria recommended to be applied to forages more susceptible to spoilage and aerobic instability such as small grain or high moisture silages. It may affect rumen fermentation since the hetero-fermentation products (acetic acid, CO_2) are used by methanogenic microorganisms as substrates for the biomethane synthesis in the rumen. In consequences, these may lead to increase in gross energy losses from diet and aggravate negative influence of ruminant production on the environment. The aim of the study was to determine the effect of *L. buchneri* on rumen methanogenesis and fermentation profile of sorghum whole crop cereal and bagasse ensiled without additive (SWCC0, SB0) and with bacterial inoculant (SWCC1, SB1). During *in vitro* rumen fermentation gas production, methanogenesis and volatile fatty acids (VFA) profile of ruminal fluid were measured. The addition of *L. buchneri* decreased the acetate concentration after 8h of *in vitro* rumen fermentation SWCC and SB silages. The material affects the acetate, propionate, butyrate, isovalerate and valerate concentration after 8h of *in vitro* rumen fermentation. Moreover, material affects the propionate, isobutyrate and butyrate concentration after 24h of fermentation. *L. buchneri* increased the level of methane after 8h fermentation of SWCC and SB. However, after 24h of fermentation *L. buchneri* decreased the concentration of methane in SWCC silage. The fermentation profile (24h) of SB silages was characterized by higher levels of methane compared to SWCC silages.

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INTRODUCTION

Livestock production represents one-third of the global anthropogenic methane emissions, similar to the fuels' production (Saunois *et al*., 2020). Between 1961 and 2018, due to the increase of ruminant production and manure excretion from various livestock categories methane emissions increased by over 51%. This trend will probably continue in the future, due to the projected rising livestock production, especially in developing countries (FAOSTAT, 2020). The largest source of CH_4 from ruminant production derives from enteric fermentation and accounts for about 47% of GHG emissions from ruminant production sector (Opio *et al*., 2013). Well-balanced diets, feed intake, feedstuffs quality, forage: concentrate ratio, energy intake, level of production, and diets supplementation with the specific feed additives may reduce methane emissions and gross energy losses during rumen fermentation (Kamra *et al.*, 2008).

Sorghum (*Sorghum* Moench) is morphologically and physiologically similar to maize being one of the most valuable forage for highly productive milking cows. However, the droughts occurring during the maize growing season may significantly reduce the yield and nutritional quality of the obtained biomass (Barbanti *et al*., 2015). Depending on the soil, climate, and hybrid, yield of sorghum forage ranges from 37-75 t/ha.

Moreover, it is characterized by an economical water management, and well-developed root system, which allows absorption of water from deeper layers of the soil during drought (Promkambut *et al*., 2010). On the other hand, sorghum can sustain in wet extremes, even flooded conditions more excellently than most of the cereal crops, especially maize. Due to the ability to grow in adverse environmental conditions sorghum is cultivated all over the world and as a feed could be an alternative to drought-sensitive maize (Ahmad Dar *et al.*, 2018). As reported by Kozłowski *et al*. (2009), the nutritional value of sorghum constitutes 80-90% of the nutritional value of maize. The moderate dietary tannin enrichment, which is present, among others in sorghum, may reduce the level of methanogenesis in the rumen (De Oliveira, 2007). The rare feature of sorghum is the ability to accumulate sucrose in the stem which makes this plant a useful source for ruminant feeding and bioethanol production (Li *et al.*, 2013; Ahmad Dar *et al*., 2018). Sugar extraction from sorghum forage results in a solid cellulosic residue (bagasse) constituting 30-35% of the fresh plant (Solomon, 2011). After pressing sorghum stalks, approximately 50% of water-soluble carbohydrates and 100% of water-insoluble structural carbohydrates remain in the bagasse (Godin *et al*., 2013). Bagasse has a wide range of applications including livestock feeding but due to low stability has to be conserved. One of the most effective methods used for bagasse preservation is ensiling (Wilk *et al.*, 2020). The most common silage inoculants: *Lactobacillus acidophilus, L. plantarum* or *Pediococcus acidilactici*-ferment water-soluble carbohydrates (WSC) in plant biomass mainly to lactic acid. One of the inoculants is *Lactobacillus buchneri* - a heterofermentative bacterium that produces acetic and lactic acid during the first weeks of fermentation. The beneficial effects of *L. buchneri* are assumed to be due to the production of acetic acid that inhibits the proliferation of some yeast species which are responsible for heat generation in aerobic conditions.

The present study aimed to determine the effect of *L. buchneri* (5×10^4 CFU/g) added during ensiling of SWCC and SB on the profile of *in vitro* rumen fermentation with particular emphasis on methanogenesis.

MATERIALS AND METHODS

Sucrosorgo 506 (*Sorghum saccharatum*) was cultivated at Research Institute Pawlowice, Poland. Part of the shredded biomass was used for juice extraction, the residue – bagasse was used to prepare silages. Sorghum whole crop cereal and sorghum bagasse were ensiled without additives (SWCC0, SB0) and with *Lactobacillus buchneri* (5×10^4 CFU/g; SWCC1, SB1). The material was ensiled in microsiloses (PVC-tubes, about 2 kg) for 180 days (at 19°C). In obtained silages the chemical composition and the quality parameters (Table 1) were determined (AOAC, 2016).

Three close-up Polish Holstein-Friesian cows were used as donors of rumen fluid. The animal diet was formulated according to ruminant feeding standards (INRA). Before the morning feeding rumen fluid was collected using the probe, blended under CO_2 and strained through four layers of cheesecloth. The samples of rumen fluid were pooled, mixed and used for analyses in six replications per each silage.

One gram of silage and ruminal fluid diluted by McDougall buffer solution (1:3) were placed in serum bottles (Sigma-Aldrich, USA). The bottles were flushed in CO_2 and sealed tightly using a capping machine. Fermentation was performed under anaerobic conditions (39°C) for 8h and 24h. After the incubation, the gas formed during fermentation was sampled for analysis. To determine the methane content of the produced gas (GP), a gas chromatograph (7890A, Agilent Technologies, US) with a thermal conductivity detection (TCD) with flame ionization detection (FID) was used. In liquid digesta samples, the pH value was measured (CP-401; Elmetron, Poland). Gas chromatograph (7890A, Agilent Technologies, US) with a FID and J&W DB-WAX column was used to determine the concentration of VFA and individual acids: acetic, propionic, isobutyric, butyric, isovaleric, valeric.

The acetic to propionic acid ratio (A:P) and the propionic to butyric acid ratio (P:B) were calculated. On the basis of the results, fermentation efficiency (FE), efficiency of fermented hexose energy to VFA energy (E1) and methane energy (E2) and the VFA utilization index (NGR) were calculated (IAEA, 1985; Czerkawski, 1986; Baran and Žitňan, 2002; Abrahamse *et al.*, 2008).

$$FE = \frac{(0.622A + 1.092P + 1.56B + iB) \times 100}{(A + P + 2B)}$$

$$E1 = \frac{\text{VFA energy}}{\text{fermented hexose energy}} = \frac{62 + 0.47P + 2B + 2V \times 100}{(100 + B + V)}$$

$$E2 = \frac{\text{methane energy}}{\text{fermented hexose energy}} = \frac{28 - 0.47P + V \times 100}{(100 + B + V)}$$

$$NGR = \frac{A + 2B + V + iB + iV}{P + V + iV + iB}$$

Where A, P, B, V, iB and iV represent respectively the molar proportions of acetate, propionate, butyrate, valerate, isobutyrate and isovalerate in the total VFA.

In vitro fermentation data for main effects were analyzed by two-ways ANOVA using Statistica 13.3 (StatSoft Inc.). Numerical data for individual treatments were analysed with one-way ANOVA. Significant differences between the groups were confirmed by Duncan's multiple range test. Differences with $P\leq0.05$ were considered as significant and $P\leq0.01$ as highly significant.

RESULTS

In all experimental treatments the amount of gas and methane increased, while the pH of the rumen fluid decreased along with the fermentation time. No differences were observed in pH value during *in vitro* fermentation of all sorghum silages. Addition of *L. buchneri* to ensiled materials increased the amount of GP and methane concentration after 8h of fermentation ($P\leq0.01$). The statistical analysis showed the effect of material and interaction of main effects on GP after 8-hour incubation that was higher in SWCC silages

compared to SB silages ($P \leq 0.01$). After 24h of fermentation there were no statistical differences between analyzed silages in pH value and GP. The statistical analysis showed the significant ($P \leq 0.01$) effect of the ensiled material on the methane concentration. However, significant ($P \leq 0.01$) interaction between the experimental factors was noted. After 24h of fermentation higher methane values were noted for SB silages compared to SWCC silages. The statistical analysis showed also the inhibitory effect of the bacterial additive ($P \leq 0.05$) on rumen methanogenesis, the lower methane concentration was found in SWCC silages ensiled with addition of inoculum (Table 2).

The concentrations of VFA increased along with incubation time in all silages (Table 3). After 8h of fermentation, material influenced the concentration of total VFA ($P \leq 0.01$), acetate, propionate, butyrate, isovalerate and valerate ($P \leq 0.05$). After 8h and 24h of SB silages incubation total VFA was lower compared to the VFA concentration obtained during SWCC fermentation. The inoculum lowered the total VFA and acetate ($P \leq 0.05$, Table 3). After 24h fermentation the material influenced

Table 1: Chemical composition and quality of SWCC and SB silages, g/kg of DM (mean±sd)

Groups	SWCC0	SWCC1	SB0*	SB1*
Dry matter, g/kg	237.04±8.30	234.06±8.33	389.03±5.52	381.55±4.74
Crude ash	50.81±0.80	53.01±1.50	33.81±0.66	35.04±0.49
Crude protein	70.12±2.64	71.82±1.90	70.93±6.81	77.91±0.60
Crude fiber	343.06±11.86	327.75±7.30	362.33±1.59	343.62±1.48
Neutral detergent fiber	627.93±15.89	615.83±9.96	707.50±6.76	682.06±4.58
Acid detergent fiber	399.34±22.81	386.51±15.22^{b}	436.32±3.28	410.95±2.13
Acid detergent lignin	51.23±1.65	53.51±5.58	61.88±1.93	55.44±2.13
Ether extract	69.75±1.32	70.36±0.77	46.84±0.06	44.63±0.77
N-free carbohydrate	466.25±13.60	477.07±6.56	486.22±9.68	499.00±0.61
Non-fiber carbohydrate	181.70±12.12	187.13±6.47	141.06±1.33	160.52±5.45
Water-soluble carbohydrate	64.02±3.10	55.55±2.98	31.72±0.61	22.61±0.51
Total VFA, g/kg of DM:	147.31±2.77	182.58±18.98	114.96±12.71	225.16±17.54
Lactic	82.92±1.29	131.59±13.19	86.15±13.68	180.30±16.04
Acetic	18.10±2.55	26.36±3.63	5.91±1.17	33.33±1.98
Butyric	23.51±2.61	14.19±0.91	19.44±0.75	9.79±0.50
Isobutyric	9.33±0.74	10.42±2.80	3.41±0.57	1.71±0.02
Formic	13.38±1.11	0.00±0.00	0.05±0.01	0.00 ±0.00
Propionic	0.00±0.00	0.02±0.00	0.00±0.00	0.02±0.00
Isovaleric	0.02±0.01	0.02±0.01	0.00±0.01	0.01±0.01
Valeric	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
NH_3-N, $\%N_{total}$	2.41±0.13	3.00±0.97	3.41±1.00	3.92±0.54
pH	3.95±0.03	3.88±0.02	4.01±0.04	3.91±0.04

* According to Wilk *et al.*, 2020.

Table 2: Gas production, methanogenesis and pH of rumen fluid after 8- and 24-hour incubation of SWCC and SB silages

Item	Gas production	Methanogenesis			pH
		mmol/kg^{X}	ml/L of Gas	ml/g DM	
		8-hour incubation			
SWCC0	1.38^{A}±0.09	1.24^{A}±0.13	119.38^{Aba}±9.20	8.96^{ABa}±0.69	6.91±0.05
SWCC1	2.48^{B}±0.27	1.50^{B}±0.16	132.60^{BCb}±7.76	9.95^{BCb}±0.58	6.93±0.04
SB0	1.78^{C}±0.23	1.21^{A}±0.14	115.22^{Aa}±7.81	8.64^{Aa}±0.59	6.91±0.07
SB1	1.57^{AC}±0.13	1.37^{AB}±0.16	137.08^{Cb}±9.76	10.28^{Cb}±0.73	6.93±0.04
P-value	0.000	0.009	0.001	0.001	0.716
		Material			
SWCC	1.93^{A}±0.61	1.37±0.20	125.99±10.65	9.45±0.80	6.92±0.04
SB	1.68^{B}±0.21	1.29±0.17	126.15±14.19	9.46±1.06	6.92±0.05
		Additive			
0	1.58^{A}±0.27	1.22^{A}±0.13	117.30^{A}±8.42	8.80^{A}±0.63	6.91±0.56
L.buchneri	2.03^{B}±0.52	1.44^{B}±0.17	134.84^{B}±8.73	10.11^{B}±0.65	6.93±0.04
		P-value			
Material	0.005	0.184	0.965	0.964	0.809
Additive	0.000	0.002	0.000	0.000	0.266
Interaction	0.000	0.359	0.236	0.236	1.000
		24-hour incubation			
SWCC0	6.85±0.55	2.38±0.18	167.45^{A}±3.62	12.56^{A}±0.27	6.77±0.05
SWCC1	6.74±0.63	2.27±0.25	151.62^{B}±4.61	11.37^{B}±0.61	6.74±0.04
SB0	6.55±0.45	2.45±0.08	165.27^{A}±4.61	12.40^{A}±0.35	6.77±0.05
SB1	7.02±0.33	2.37±0.30	168.62^{A}±5.40	12.53^{A}±0.24	6.77±0.05
P-value	0.420	0.590	0.000	0.000	0.651
		Material			
SWCC	6.77±0.57	2.33±0.22	159.53^{A}±10.20	11.97^{A}±0.77	6.75±0.05
SB	6.78±0.45	2.41±0.21	166.94^{B}±5.10	12.47^{B}±0.29	6.77±0.05
		Additive			
0	6.70±0.51	2.42±0.14	166.36^{a}±4.11	12.48^{a}±0.31	6.77±0.05
L.buchneri	6.85±0.50	2.32±0.27	160.12^{b}±11.04	11.95^{b}±0.75	6.76±0.05
		P-value			
Material	0.939	0.366	0.005	0.001	0.411
Additive	0.486	0.313	0.014	0.012	0.620
Interaction	0.134	0.859	0.001	0.001	0.411

^{x} mmol/kg undiluted ruminal fluid.

Table 3: VFA profile of ruminal fluid after 8- and 24-hour incubation of SWCC and SB silages

Item	Total VFA	A[z]	P[z]	iB[z]	B[z]	iV[z]	V[z]
			8-hour incubation				
SWCC0	76.26^{A}±4.34	50.17^{A}±3.96	16.61±1.37	0.51±0.03	7.13±0.50	0.75±0.04	0.90±0.10
SWCC1	72.18^{AB}±3.70	45.93^{AB}±3.71	16.76±0.74	0.48±0.05	7.14±0.22	0.74±0.05	0.93±0.06
SB0	70.43^{AB}±3.41	46.13^{AB}±2.42	15.56±0.70	0.47±0.03	6.55±0.61	0.69±0.07	0.84±0.07
SB1	68.19^{B}±2.67	43.92^{B}±2.79	15.52±1.40	0.47±0.01	6.58±0.66	0.67±0.07	0.84±0.04
P-value	0.007	0.029	0.122	0.207	0.099	0.079	0.079
			Material				
SWCC	74.22^{A}±4.40	48.05^{a}±4.58	16.69^{a}±1.05	0.49±0.04	7.14^{a}±0.37	0.75^{a}±0.04	0.92^{a}±0.08
SB	69.31^{B}±3.15	45.02^{b}±2.74	15.54^{b}±1.06	0.47±0.02	6.56^{b}±0.61	0.68^{b}±0.07	0.84^{b}±0.05
			Additive				
0	73.34^{a}±4.81	48.15^{a}±3.77	16.08±1.17	0.49±0.03	6.84±0.61	0.72±0.06	0.87±0.09
L.buchneri	70.18^{b}±3.72	44.92^{b}±3.30	16.14±1.25	0.48±0.03	6.86±0.55	0.70±0.07	0.89±0.07
			P-value				
Material	0.003	0.035	0.019	0.094	0.015	0.014	0.016
Additive	0.043	0.026	0.904	0.342	0.933	0.496	0.442
Interaction	0.537	0.459	0.835	0.342	0.963	0.784	0.627
			24-hour incubation				
SWCC0	114.43^{ab}±5.44	77.78±6.74	23.88^{AB}±1.33	0.73^{ab}±0.08	9.65^{AB}±0.91	1.05±0.16	1.15±0.11
SWCC1	118.52^{a}±3.26	74.61±6.64	25.97^{B}±1.17	0.78^{b}±0.06	10.59^{B}±1.17	1.17±0.09	1.23±0.09
SB0	110.37^{b}±7.82	78.59±4.31	24.42^{AB}±0.81	0.67^{a}±0.05	8.32^{A}±0.82	1.06±0.10	1.31±0.15
SB1	108.52^{b}±7.54	73.90±6.68	22.98^{A}±0.70	0.70^{a}±0.07	8.61^{A}±1.00	1.03±0.17	1.11±0.07
P-value	0.050	0.488	0.000	0.029	0.002	0.316	0.196
			Material				
SWCC	116.48^{a}±4.78	78.18±5.41	24.92^{A}±1.62	0.76^{A}±0.07	10.12^{A}±1.11	1.11±0.14	1.19±0.10
SB	109.45^{b}±7.39	74.26±6.36	23.70^{B}±1.05	0.68^{B}±0.06	8.46^{B}±0.88	1.04±0.13	1.11±0.11
			Additive				
0	112.40±6.76	76.19±6.59	24.15±1.09	0.70±0.07	8.99±1.08	1.05±0.13	1.13±0.12
L.buchneri	113.52±7.61	76.25±5.89	24.47±1.81	0.74±0.07	9.60±1.46	1.10±0.15	1.17±0.10
			P-value				
Material	0.013	0.135	0.009	0.009	0.000	0.232	0.087
Additive	0.667	0.983	0.450	0.124	0.142	0.410	0.394
Interaction	0.261	0.765	0.001	0.751	0.438	0.222	0.299

[x] mmol/kg undiluted ruminal fluid; [z] mol/100 mol of total VFA concentration.

Table 4: VFA indexes of ruminal fluid after 8- and 24-hour incubation of SWCC and SB silages

Item	A:P	P:B	NGR	EE (%)	EI (%)	E2 (%)
			8-hour incubation			
SWCC0	3.04±0.35	2.35±0.33	3.57±0.38	75.26±0.82	80.24±0.76	18.22±0.87
SWCC1	2.75±0.25	2.35±0.12	3.30±0.24	76.01±0.76	80.89±0.99	17.62±0.59
SB0	2.97±0.14	2.39±0.18	3.49±0.16	75.38±0.38	80.31±0.37	18.09±0.38
SB1	2.86±0.37	2.40±0.46	3.40±0.43	75.78±0.93	81.30±0.76	17.81±1.06
P-value	0.360	0.987	0.529	0.302	0.075	0.542
			Material			
SWCC	2.89±0.33	2.35±0.24	3.43±0.33	75.64±0.85	80.77±1.01	17.92±0.77
SB	2.91±0.27	2.39±0.33	3.44±0.31	75.58±0.71	80.60±0.65	17.95±0.77
			Additive			
0	3.00±0.26	2.37±0.26	3.53±0.28	75.32±0.61	80.28^{a}±0.57	18.15±0.65
L. buchneri	2.80±0.31	2.37±0.32	3.35±0.34	75.90±0.82	81.10^{b}±0.87	17.72±0.82
			P-value			
Material	0.864	0.719	0.935	0.859	0.585	0.929
Additive	0.108	0.987	0.194	0.075	0.015	0.178
Interaction	0.468	0.968	0.511	0.574	0.443	0.620
			24-hour incubation			
SWCC0	3.28±0.45	2.49^{b}±0.25	3.75±0.41	74.65±1.07	79.11±1.34	18.58±0.88
SWCC1	3.04±0.27	2.47^{b}±0.25	3.54±0.20	75.20±0.72	79.89±1.20	18.19±0.49
SB0	3.05±0.21	2.96^{a}±0.27	3.45±0.24	75.01±0.59	79.00±0.49	17.96±0.55
SB1	3.22±0.32	2.70^{ab}±0.30	3.64±0.34	74.70±0.69	78.89±0.73	18.39±0.72
P-value	0.493	0.016	0.398	0.587	0.323	0.442
			Material			
SWCC	3.16±0.37	2.48^{A}±0.24	3.64±0.33	74.92±0.91	79.50±1.28	18.38±0.71
SB	3.14±0.27	2.83^{B}±0.30	3.55±0.30	74.85±0.64	78.95±0.60	18.17±0.65
			Additive			
0	3.16±0.35	2.72±0.35	3.60±0.36	74.83±0.84	79.39±1.08	18.27±0.77
L. buchneri	3.13±0.30	2.59±0.29	3.59±0.27	74.95±0.72	79.05±0.97	18.29±0.60
			P-value			
Material	0.886	0.005	0.452	0.834	0.192	0.455
Additive	0.789	0.218	0.934	0.706	0.421	0.943
Interaction	0.142	0.277	0.131	0.198	0.287	0.155

the total VFA ($P \leq 0.05$), propionate, isobutyrate and butyrate ($P \leq 0.01$). The statistical analysis showed the influence of the interaction of the main factors on propionate ($P \leq 0.01$).

L. buchneri increased ($P \leq 0.05$) efficiency of fermented hexose energy to VFA energy, after 8h of fermentation, while material influenced the P:B ratio ($P \leq 0.01$) after 24h of fermentation (Table 4).

DISCUSSION

The high level of CF in mature forage contributes to increase concentration of acetate in the rumen. The addition of *L. buchneri* decreased the level of CF in silages, which affected acetate during *in vitro* fermentation. Decrease in CF concentration and a high level of starch (NFE) leads to increased propionate concentration and decrease valerate and isovalerate concentration in rumen (Van Gastelen *et al.*, 2015). The differences in propionate, isovalerate and valerate in materials are reflected in E1.

Along with increasing starch content, which is a component of NFE, in the diet, the population of ruminal protozoa increases. Protozoa produce butyric acid as an end product of carbohydrate fermentation (Brossard *et al*., 2004). *L. buchneri* increased the content of NFE in SB but it did not affect butyrate concentration in the rumen. The contribution of butyrate to total VFA is clearly lower than the optimal physiological proportions of A:P:B in the rumen fluid (65:20:15). The A:P ratio below 3:1 in the rumen content contributes to the low-fat milk. The VFA utilization index (NGR) was expressed as the non-glucogenic VFA to glucogenic VFA ratio. The NGR index is associated with effects on methane production, milk composition, and energy balance (Morvay *et al*., 2011). Glucogenic propionate causes energy deposition in the body tissues, while nonglucogenic acetate and butyrate are sources of LCFA. NGR below 3.0 increases the risk of low-fat milk. Low values of NGR (also confirmed in this study: 3.3-3.8) indicate low energy loss in ruminal gases (Abrahamse *et al.*, 2008). The VFA profile determines hydrogenesis in the gastrointestinal tract and thus affects the level of methane production as an excess hydrogen absorber (Monteny *et al.*, 2006). Propionate, as an alternative to methane, also captures metabolic hydrogen reducing the production of methane from a unit of fermented organic mass, thereby increasing the cow milk yield (Mills *et al.*, 2001; Janssen, 2010). Higher propionate level found after 24h fermentation of SWCCs explains the lower methane concentration compared to SBs. The ambiguous effect of LAB in ruminant nutrition on methanogenesis was reported by Jeyanathan *et al.* (2016), Astuti *et al.* (2018), Varnava *et al.* (2017). Ellis *et al.* (2016) who found their effectiveness also depends on plant species. Kupryś-Caruk (2017) reported that the additive of heterofermentative LAB increases CH_4 production. *L. buchneri* increased methane production during rumen fermentation of SWCC and SB after 8h of fermentation, but it does not increase metabolizable energy losses (E2). After 24h of ruminal fermentation, methane concentration in silages with inoculum were lower compared to the control silages and more methane was synthesized during fermentation of SB than SWCC silages which may be inducted by higher DM content (Podkówka and Podkówka, 2011).

The products of lactic acid fermentation are used by methane bacteria as substrates for the biomethane production. High concentration of acetate, being the fermentation product of heterofermentative *L. buchneri*, in silages intended for bioplants could enhance methane formation (Kalač, 2011; Podkówka and Podkówka, 2011). In order to maximize methanogenesis, DM of silage should be about 30-35% and starch concentration of 30% DM. However, earlier harvesting, due to immature grain and insufficient starch content, and also delayed sorghum harvesting, due to the increase in lignocellulosic compounds contents which are difficult to decompose in the process of methane fermentation, reduces the yield of biogas.

The FE index is used to evaluate the effect of feed additives on rumen fermentation through microbial metabolism modulation. The FE was similar in all silages (about 75%). In correlation to these results also the E2 were similar in all silages (about 18%).

Conclusions: *L. buchneri* affects the process of methanogenesis. The 8-h rumen fermentation of both SWCC and SB showed a clear effect of inoculum on the increase in methane synthesis. However, the amount of methane produced was lower after 24-h incubation of both materials ensiled with *L. buchneri.* The rumen fermentation profile, especially higher total VFA and acetate in SWCC is a consequence of the greater content of easily degradable components in this material. *L. buchneri* can reduce the methanogenesis of high-fibrous feed materials without adversely affecting rumen fermentation.

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REFERENCES

Abrahamse PA, Vlaeminck B, Tamminga S, *et al.,* 2008. The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. J Dairy Sci 91:4778-92.

Ahmad Dar R, Ahmad Dar E, Kaur A, *et al*., 2018. Sweet sorghum-a promising alternative feedstock for biofuel production. Renew Sustain Energy Rev 82:4070-90.

AOAC, 2016. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Astuti WD, Wiryawan KG, Wina E, *et al.*, 2018. Effects of selected *Lactobacillus plantarum* as probiotic on *in vitro* ruminal fermentation and microbial population. Pak J Nutr 17:131-9.

Baran M and Žitňan R, 2002. Effect of monensin sodium on fermentation efficiency in sheep rumen. Arch Tierzucht 45:181-5.

Barbanti L, Sher A, Di Girolamo G, *et al*., 2015. Growth and physiological response of two biomass sorghum (*Sorghum bicolor* L. Moench) genotypes bred for different environments, to contrasting levels of soil moisture. Ital J Agron 10:208-14.

Brossard L, Martin C, Chaucheyras-Durand F, *et al*., 2004. Protozoa involved in butyric rather than lactic fermentative pattern during latent acidosis in sheep. Reprod Nutr Dev 44:195-206.

Czerkawski JW, 1986. An introduction to rumen studies. Pergamon Press, Oxford, pp:221-2.

De Oliveira SG, Berchielli TT, Santos Pedreira M, *et al*., 2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. Anim Feed Sci Technol 135:236-48.

Ellis JL, Bannink A, Hindrichsen IK, *et al.*, 2016. The effect of lactic acid bacteria included as a probiotic or silage inoculant on *in vitro* rumen digestibility, total gas and methane production. Anim Feed Sci Technol 211:61-74.

FAOSTAT, 2020. Food and Agriculture Organization of the United Nations, 2020. Retrieved from http://www.fao.org/aostat/en/ #home

Godin B, Lamaudière S, Agneessens R, *et al*., 2013. Chemical composition and biofuel potentials of a wide diversity of plant biomasses. Energ Fuel 27:2588-98.

International Atomic Energy Agency, 1985. Laboratory training manual on the use of nuclear techniques in animal nutrition. Technical Report Series, Vienna pp:301.

Janssen PH, 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Technol 160:1-22.

Jeyanathan J, Martin C and Morgavi DP, 2016. Screening of bacterial direct-fed microbials for their antimethanogenic potential *in vitro* and assessment of their effect on ruminal fermentation and microbial profiles in sheep. J Anim Sci 94:739-50.

Kalač P, 2011. The required characteristics of ensiled crops used as a feedstock for biogas production: a review. J Agrobiol 28:85-96.

Kamra DN, Patra AK, Chatterjee PN, *et al*., 2008. Effect of plant extracts on methanogenesis and microbial profile of the rumen of buffalo: a brief overview. Aust J Exp Agri 48:175-8.

Kozłowski S, Zielewicz W, Potkański A, *et al*., 2009. Effect of chemical composition of sugar sorghum and the cultivation technology on its utilization for silage production. Acta Agron Hung 57:67-78.

Kupryś-Caruk MP, 2017. The effect of microbial silage additives on biogas production from perennial energy grasses. J Agric Eng 62:68-71.

Li J, Li S, Han B, *et al*., 2013. A novel cost-effective technology to convert sucrose and hemicelluloses in sweet sorghum stalks into ethanol. Biotechnol Biofuels 6:174.

Mills JAN, Dijkstra J, Bannink A, *et al*., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating cow: model development, evaluation, and application. J Anim Sci 79:1584-97.

Monteny GJ, Bannink A and Chadwick D, 2006: Greenhouse gas abatement strategies for animal husbandry. Agric Ecosyst Environ 112:163-70.

Morvay Y, Bannink A, France J, *et al*., 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. J Dairy Sci 94: 3063-80.

Opio C, Gerber P, Mottet A, *et al*., 2013. Greenhouse gas emissions from ruminant supply chains – A global life cycle assessment. Food and Agriculture Organization of the United Nations (FAO), Rome.

Podkówka Z and Podkówka L, 2011. Chemical composition and quality of sweet sorghum and maize silages. J Cent Eur Agric 12:294-303.

Promkhambut A, Younger A, Polthanee A, *et al*., 2010. Morphological and physiological responses of sorghum (*Sorghum bicolor* L. Moench) to water logging. Asian J Plant Sci 9:183-93.

Saunois M, Stavert AR, Poulter B, *et al*., 2020. The global methane budget 2000–2017. Earth Syst Sci Data 12:1561-623.

Solomon S., 2011. Sugarcane By-Products Based Industries in India. Sugar Tech 13:408-16.

Van Gastelen S, Antunes-Fernandes EC, Hettinga KA, *et al*., 2015. Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage or corn silage-based diets. J Dairy Sci 98:1915-27.

Varnava KG, Ronimus RS and Sarojini V, 2017. A review on comparative mechanistic studies of antimicrobial peptides against archaea. Biotechnol Bioeng 114:2457-73.

Wilk M, Król B, Słupczyńska M, *et al*., 2020. Effect of *Lactobacillus buchneri* on the nutritive value of Sucrosorgo 506 bagasse silage. J Anim Feed Sci 29:158-66.