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Changes in the In Vitro Ruminal Fermentation of Diets for Dairy Cows Based on Selected Sorghum Cultivars Compared to Maize, Rye and Grass Silage

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Abstract: An in vitro experiment was conducted to determine the impact of silage produced from selected varieties of sorghum on the microbial fermentation profile of cows' ruminal fluid. To determine the main microbial fermentation products, ruminal fluid samples were obtained from Polish Holstein–Friesian cows. Serum bottles were filled with 80 mL of ruminal samples, and 1 g of one of the following substrates was added: corn silage (CS), grass silage (GS), rye silage (RS), sorghum silage (sweet) (SS1), sorghum silage (grain) (SS2) or sorghum silage (dual-purpose) (SS3). The serum bottles were flushed with CO_2 and fermented for 8 and 24 h at 39 °C. After incubation, the obtained gas and rumen fluid were then analysed to determine the methane and volatile fatty acid (VFA) contents using gas chromatography. The use of sorghum silage (SS) resulted in a decrease in the total concentration VFA concentration in the ruminal fluid compared with the use of other silages, especially GS. Moreover, the ruminal fluid contained a lower molar proportion of propionic and butyric acids when SS was used compared with CS. The butyric acid proportion was higher in SS samples than in RS samples. The differences in chemical composition between sorghum varieties did not influence the rumen VFA concentration or profile. A decrease in gas production, but without effects on methanogenesis, was observed when SS was used compared with GS and CS. The analysis demonstrates the physiological processes of fermentation in the rumen, as evidenced by the products of microbial fermentation. The main advantage is that the addition of SS, irrespective of the plant variety, reduced fermentation gas production in the ruminal fluid compared with CS. The silage of the analyzed sorghum varieties may be used in the diets of dairy cows as a substitute for corn and grass silages.

Keywords: rumen fermentation; sorghum silage; corn silage; rye silage; grass silage; methane; volatile fatty acids

1. Introduction

Changes in environmental and climate conditions have been observed in European countries, including Poland, for several years, leading to the intensification of research on alternative feed components for animal production [1]. Furthermore, climate changes have broad and far-reaching effects on animal and plant production, crop biodiversity and water availability [2]. Therefore, it is worth searching for new solutions, such as

the utilization of less popular but prospective crops, including sorghum (*Sorghum* sp.). Sorghum, a multipurpose crop cultivated for grain, sweet stem, forage and broomcorn, is the fifth most important cereal in the world, with high potential applications in food production [3]. Nowadays, sorghum is also cultivated in some European countries [4]. Sorghum, unlike maize, is resistant to drought and is widely cultivated in subtropical climates where water availability is a limiting factor in crop production [5–7]. Moreover, it is characterized by a higher tolerance to seasonal rises in temperature and less fertile soils than corn [8,9]. Its high crude protein content and moderate structural carbohydrate and lignin content make the sorghum plant attractive as a component of animal fodder [10,11]. Because of its lower energy content compared to corn silage, sorghum silage could be used for feeding heifers and dry cows [12,13]. However, the sweet sorghum variety (*Sorghum saccharatum*) is characterized by a high energy content due to the high content of water-soluble carbohydrates [9]. Cattani et al. has shown that a diet based on sorghum silage could be supplemented with corn starch and used instead of corn silage to ensure high milk yield in dairy cows [14].

However, sorghum's morphology, especially its large stems, makes curing difficult and negatively affects hay production from the plant. Therefore, harvesting sorghum crops for silage is considered the most suitable alternative for its conservation, to ensure year-round feed supply for ruminants [15]. Furthermore, sorghum silage made from different varieties have different chemical compositions and energy values, so their nutritional value must be determined before they are included in the diet [16,17]. Recent studies by Khosravi et al. reported beneficial effects of replacing corn silage with sorghum silage in the diets of lactating cows in terms of the antioxidant capacity and fatty acid profile of their milk [18].

It is essential to perform rumen fermentation in order to assess the full range of possible uses for the feed of dairy cows. In the literature, there are no comparative analyses of in vitro fermentation of the rumen content with the addition of different sorghum silages and corn, rye, or grass silages. Additionally, there is little data on ruminants fed dual-purpose sorghum (used as fodder and grain).

Therefore, we formulated a hypothesis that the silage of sweet, grain and dual-purpose sorghum used in the experiment would not have a detrimental influence on the microbial fermentation of ruminal fluid, and that some parameters might improve. The use of corn, rye, and grass silages as control substrates would compare the profile and efficiency of microbial fermentation and provide guidance on how to replace individual components of cattle diets with sorghum silage. Therefore, the objective of the present in vitro experiment was to elucidate the impact of silage from selected sorghum varieties compared to corn, rye, and grass silages on the rumen fermentation, especially the VFA concentration and methane production, as well as the total fermentation gas production, fermentation efficiency, and microbial cell yield.

2. Materials and Methods

2.1. Animals

The experiment was conducted on 10 Polish black-and-white Holstein–Friesian variety cows. The study was conducted in Wroclaw, Poland (latitude: 51°6′28.3788″ N, 17°2′18.7368″ E). The animals were kept in a free-stall system and fed a total mixed ration (TMR) diet, the composition of which is presented in Table 1. The cows for the experiment were selected, taking into consideration their age and milk yield from previous lactation. Ruminal fluid was sampled two months after the second calving of cows with an average body weight of about 694 kg. The sample collections took place in spring (April) over the period of 7 days. The feed ration for milking cows in the mid-lactation period producing 25 kg of milk/d was composed according to the French INRA (Instytut National de la Recherche Agronomique) standard [19]. The animals had unlimited access to water, were kept in good hygienic conditions, and had no symptoms of the disease.

Table 1. Composition and nutritional value of cows' diet (g/kg DM).

Diet Composition	
Feedstuff	g/kg DM
Maize silage	492.0
Alfalfa forage	290
Alfalfa hay silage	87.9
Rapeseed meal	35.1
Meadow hay	26.4
Barley ground	26.4
Lupin ground	17.6
Triticale ground	17.6
Premix	7.0
DM (kg)	20,32
Forage-concentrate ratio	90:10
Diet Nutritional Value	
Ingredient	g/kg DM
OM	930
CP	138
NDF	439
ADF	244
ADL	47
Starch	162
WSC	60
Ca abs	2.5
P abs	2.0
FV	0.92
UFL	0.85
PDIN	87
PDIE	79
PDIA	29

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; WSC, water-soluble carbohydrates; abs, absorption; FV, fill value; UFL, unit for lactation; PDIN, the sum of microbial protein that could be synthesized from available N and undegraded dietary protein in the rumen but is digestible in the small intestine; PDIE, the sum of microbial protein that could be synthesized from available energy and undegraded dietary protein in rumen but is digestible in the small intestine; PDIA, undegraded dietary protein in rumen, but is digestible in the small intestine.

2.2. Plant Materials

The materials for the study were forages of different sorghum varieties from an experimental plot in Pawlowice (51°17′32″ N, 17°11′72″ E) belonging to the Institute of Agroecology and Plant Production of Wroclaw University of Environmental and Life Sciences: sucrosorgo 506, grain sorghum type NK251 and dual-purpose sorghum of Sweet the Caroline variety. Sucrosorgo 506 is a late-maturing triple-cross sweet sorghum hybrid. Based on previous research, this hybrid produces a high biomass yield with very low panicle content (no grain) in the moderate climate of Central Europe [20]. The plants reach heights of 250–350 cm. NK251 (12GS9010) is a very early grain sorghum hybrid with high drought resistance recommended especially for arid regions; it has high panicle and grain content and good yielding standability. The plants reach heights of 80–100 cm. Sweet Caro-

line is a middle maturity double-purpose hybrid characterized by compact, leafy plants that with good plant stability; it has very high grain and whole plant biomass yield and high energetic value. After 120 days of growth, all sorghum plants were harvested and chopped with a bowl chopper (Krag, Poland) into pieces about 20–25 mm long. The plant material was pressed to remove air and ensiled at laboratory scale in microsiloses (PVC tubes, about 2 kg) for 180 days (at 19 °C) without silage additives. All silages were prepared in 6 replications. After 180 days, the ensiled material was analysed chemically. The obtained research materials were compiled with 3 forages commonly used in ruminant production ensiled on the farm-scale: grass (seed mixture: perennial ryegrass (*Lolium perenne* L.), 30%; tall fescue (*Festuca arundinacea* Schreb.), 30%; orchard grass (*Dactylis glomerata* L.), 20%; timothy grass (*Phleum pratense* L.), 10%; meadow fescue (*Festuca pratensis* Huds.), 10%; winter rye (*Secale cereale* L.) and corn (*Zea mays* L.) silages. All the plant materials were harvested in an optimal and commonly recommended phase of vegetation with regard to yield and nutritional value of biomass: grass forage 60 days after the growing season started (first cut, beginning of heading, short chaff, wilted in the field, 35% DM), whole-crop winter rye forage 227 days after sowing (milk-dough stage, normal conditions) and corn 148 days after sowing (dough-flint stage, short chaff, normal conditions). All the forages were chopped with a chaff-cutter into pieces about 20–25 mm long. The plant forages were ensiled on a concrete base without silage additive. Corn, grass and rye silages were dried, and chemical analyses were performed.

2.3. Chemical Analyses

The basic chemical components of the representative plant silage samples were determined as follows: dry matter using weight method and drying the sample in a forced-air oven at 105 °C (DM; method 934.01 of Association of Official Agricultural Chemists—AOAC) [21]. Crude protein was calculated based on nitrogen content determined using a Kjeltec 2300 Foss Tecator apparatus (Häganäs, Sweden) and by multiplying the nitrogen content by 6.25; (CP; Kjeldahl method, method 984.13 of AOAC) [21]. Ether extract by continuous extraction performed on dried samples in a Soxhlet extractor, without previous acid hydrolysis (EE; method 920.39 of AOAC) [21]. Crude fibre, according to the Henneberg–Stohmann method, by subsequent hydrolysis of the feed sample with acid and base solution using an Fibertec Tecator (Häganäs, Sweden) apparatus (CF; method 978.10 of AOAC) [21]. Crude ash was determined by sample combustion in a muff oven at 550 °C (method 942.05 of AOAC) [21]. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were determined using an Fibertec Tecator (Häganäs, Sweden) apparatus (NDF; method of Holst, 1973 [22]) and acid detergent fibre (ADF; method 973.18 of AOAC [21]). The net energy of lactation (NEL) in unit for lactation (UFL) was estimated according to the INRA feeding system [19]. The nonstructural carbohydrate (NSC) content was calculated according to the National Research Council [23] as follows: $100 - (Ash + CP + EE + NDF)$, and nitrogen-free extractives (NFE) were calculated as $100 - (Ash + CP + EE + CF)$, with ash, CP, EE, CF and NDF contents expressed as % of DM. The chemical composition of plant substrates is presented in Table 2.

2.4. In Vitro Fermentation

The rumen content of 10 cows was sampled using a probe 2 h after morning feeding with TMR; 500 mL of ruminal fluid was taken from each cow, filtered through 2 layers of cheesecloth, and then placed as inoculum in twelve 125 mL serum bottles (20 mL in each bottle). The obtained material was mixed with buffer solution [24] in a 1:3 ratio and homogenized. Incubation was performed in a shaking water bath at 39 °C for 2 incubation periods: 8 and 24 h [25]; 6 bottles of ruminal fluid from each cow were used for 8 h incubation and 6 bottles for 24 h incubation. For each cow and each fermentation period, 6 samples were formulated based on the supplementary substrate (1 g per bottle): corn silage (CS), grass silage (GS), rye silage (RS), sweet sorghum silage (SS1), grain sorghum silage (SS2) and dual-purpose sorghum silage (SS3). In summary, 120 samples were prepared to

perform the in vitro fermentation, 60 samples for each fermentation period. The bottles were flushed with carbon dioxide and sealed using a manual crimper. The incubation was performed in a shaking water bath at 39 °C for 8 and 24 h [25].

Table 2. Chemical composition of substrates used for in vitro fermentation (% of DM).

Item	CS	GS	RS	SS1	SS2	SS3
Dry matter (DM) (% of fresh)	35.04	33.53	32.45	23.16	37.67	23.52
Crude protein	8.14	13.93	10.93	7.09	6.72	6.36
Ether extract	2.41	2.75	1.53	1.65	3.35	1.42
Crude fibre	21.51	26.05	35.85	33.36	25.90	36.92
Neutral detergent fibre	48.16	45.05	64.42	64.37	49.83	70.42
Acid detergent fibre	28.61	28.71	39.38	38.81	28.04	43.19
Ash	3.73	10.63	9.74	5.42	6.17	5.29
Nonstructural carbohydrates	37.56	27.64	13.38	21.48	33.93	16.51
Nitrogen free extractives	64.21	46.64	41.95	52.48	57.86	50.01
Gross energy (MJ/kg DM)	5.37	6.74	6.14	4.80	7.27	4.31

CS, corn silage; GS, grass silage; RS, rye silage; SS1, sorghum silage (sweet sorghum); SS2, sorghum silage (grain sorghum); SS3, sorghum silage (dual-purpose sorghum).

2.5. Analysis of Fermentation Products

The analysis of microbial metabolites, such as VFAs and methane, was carried out basically as described above [26]. After the incubation, the headspace pressure created by fermentation gas in the serum bottles was measured. The gas and liquid samples were obtained, and liquid samples were analyzed; pH value was measured using a CP-401 pH meter (Elmetron, Zabrze, Poland) with an EPP-3 electrode and temperature sensor. Then the solution was centrifuged with cooling ($2800\times$ g for 20 min) and formic acid was added (0.1 mL/2 mL solution) to stop the fermentation process. Liquid samples were analyzed on a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector and Agilent J&W DB-WAX column, with helium as the carrier gas (flow: 25 mL/min), to determine the particular VFA concentrations: acetic, propionic, isobutyric, butyric, isovaleric and valeric acids [27]. The fatty acids in the analysed samples were identified and their concentration was determined by comparing the retention time and peak area with a VFA Supelco standard using the ChemStation v. 4.01 software (Agilent Technologies, Santa Clara, CA, USA). The total VFA concentration was calculated as the sum of the individual VFA concentrations in the ruminal fluid. Based on the obtained results, the proportion of each VFA in the total VFA amount was also calculated. The fermentation gas was then analyzed to determine the methane content using an Agilent 7890A gas chromatograph equipped with a thermal conductivity detector (TDC) and a flame ionization detector (FID); two Supelco columns, Porapak Q and HayeSep Q (Supelco, Bellefonte, PA, USA); and a 5A molecular sieve, with helium as the carrier gas (flow: 25 mL/min) [28]. Based on the CH_4 percentage in the headspace gas volume and the gas pressure measurements, the molar CH_4 concentration was calculated using the Clapeyron equation (ideal gas equation).

2.6. Calculations and Statistical Analysis

Using the concentration and profile of VFAs, their fermentation efficiency index (FE) was calculated using the equation worked out by Baran and Žitňan [29]: FE = (0.622A + 1.092P + 1.56B)100/(A + P + 2B) [29], where A, P and B are the molar proportions (mol %) of acetic, propionic and butyric acid in the total VFA amount. Additionally, the cell yield index in the rumen was calculated using the equation developed by Chalupa [30]: CY = (A + P + B + V) × 0.03, where A, P, B and V are the concentration (mmol/L of ruminal fluid) of acetic, propionic, butyric and valeric acid, respectively. The CY index was calculated based on 30 g of microbial cells per mol of volatile fatty acid (g/L).

Statistical analysis of the results was performed using two-factor analysis of variance (ANOVA) in Statistica PL v.10 (StatSoft, Tulsa, OK, USA), according to the following equation:

$$Y_{ijk} = \mu + SB_i + T_j + (SB \times T)_{ij} + e_{ijk} \tag{1}$$

where Y_{ijk} is the dependent variable under examination, μ is the overall mean, SB_i is the effect of the substrate, T_j is the effect of the fermentation period, $SB \times T$ is the fixed effect of the interaction between substrate and fermentation period, and e_{ijk} is the error term. Differences between means were considered statistically significant at $p < 0.05$ [31].

3. Results

Chemical analyses showed that, between the sorghum silage samples (SS), SS3 was the richest in crude fibre, NDF and ADF, followed by SS1, whereas SS2 was the richest in NSC (Table 2). However, these differences in chemical composition between sorghum varieties did not influence the rumen VFA concentration or profile. Nevertheless, the use of SS resulted in a decrease in production the production of VFA in the ruminal fluid as compared with the use of other silages, especially GS ($p < 0.01$) (Table 3). Moreover, the ruminal fluid contained a lower proportion of propionic acid when SS was used compared with CS ($p < 0.05$). CS caused a lower proportion of acetic acid in ruminal samples ($p < 0.01$) compared with other substrates. Furthermore, the butyric acid molar proportion was lower in SS than CS samples, but higher than in RS ($p < 0.01$).

Table 3. Influence of silage substrates on VFA profile and pH in ruminal fluid.

	Incubation Time (h)	Substrates						SEM	*p* Value [1]		
		CS	GS	RS	SS1	SS2	SS3		SB	T	SB × T
Total VFA [2]	8	215.1	265.3	232.9	186.4	190.8	190.8	7.095	<0.01	<0.01	0.59
	24	339.2	361.9	324.1	325.1	321.2	296.3				
Individual VFA, mol/100 mol											
Acetic acid	8	63.08	66.73	65.60	66.94	65.69	63.76	0.255	<0.01	<0.05	0.36
	24	63.23	67.41	66.44	67.32	66.85	67.08				
Propionic acid	8	21.71	20.11	20.84	18.64	20.41	21.08	0.224	<0.05	0.35	0.40
	24	20.90	19.97	21.23	19.81	18.48	19.67				
Isobutyric acid	8	0.66	0.65	0.79	0.65	0.53	0.61	0.012	<0.01	<0.01	0.24
	24	0.75	0.74	0.82	0.69	0.71	0.67				
Butyric acid	8	10.83	9.20	8.62	10.30	10.01	10.63	0.143	<0.01	<0.05	0.05
	24	11.58	8.58	7.79	9.14	10.44	9.36				
Isovaleric acid	8	1.37	1.05	1.38	1.26	1.29	1.13	0.022	<0.01	<0.05	0.84
	24	1.54	1.24	1.47	1.30	1.37	1.30				
Valeric acid	8	1.77	1.83	2.16	1.67	1.52	1.88	0.054	<0.05	<0.01	0.98
	24	1.53	1.68	1.82	1.32	1.30	1.47				
A:P	8	2.94	3.35	3.16	4.22	3.27	3.21	0.084	0.08	0.98	0.35
	24	3.08	3.39	3.14	3.43	3.55	3.39				
P:B	8	2.08	2.24	2.45	1.85	2.07	2.02	0.041	<0.01	0.31	0.09
	24	1.84	2.37	2.75	2.18	1.84	2.14				
pH	8	6.53	6.55	6.71	6.66	6.65	6.73	0.014	<0.01	<0.05	0.77
	24	6.52	6.54	6.60	6.60	6.62	6.61				

CS, corn silage; GS, grass silage; RS, rye silage; SS1, sorghum silage (sweet sorghum); SS2, sorghum silage (grain sorghum); SS3, sorghum silage (dual-purpose sorghum); SEM, standard error of the mean; [1] SB, effect of the substrate; T, effect of time; SB × T, the interaction effect of substrate and time; [2] mmol/L of undiluted ruminal fluid; A:P, acetic to propionic acid ratio; P:B, propionic to butyric acid ratio.

The substrates also influenced the proportion of isoacids in our research ($p < 0.01$) (Table 3). In the SS samples, isobutyric acid was at a relatively low level, but isovaleric acid was at an average level compared with the other samples. RS substrate resulted in increased production of both isobutyric and isovaleric acids compared with other substrates, whereas CS addition resulted in the highest concentration of isovaleric acid in the ruminal fluid. The pH of ruminal fluid with SS, especially SS3 ($p < 0.05$), was higher compared with CS and GS, most likely as a result of lower VFA concentration (Table 3).

No influence of the analyzed substrates on the proportion of acetic to propionic acid (A:P) in the ruminal fluid was observed, but the proportion of propionic to butyric acid (P:B) changed significantly ($p < 0.01$) (Table 3). After the addition of SS to the incubated digesta, the P:B ratio was lower in comparison with rye or grass silage. SS2 substrate caused a similar P:B ratio as CS substrate.

In our study, FE observed in ruminal fluid containing SS was on an average level compared to other samples, the highest value of which was observed in CS ($p < 0.01$) (Table 4). On the other hand, FE in RS decreased compared with that in SS samples ($p < 0.05$). Moreover, the use of SS as substrate in the ruminal fluid decreased the production of gas ($p < 0.01$), especially in comparison with GS and CS samples (Table 4). However, methane production was similar in all samples except for RS, which was lower compared with CS ($p < 0.01$), GS ($p < 0.05$) and SS2 ($p < 0.05$) (Table 4).

Table 4. Influence of silage substrates on fermentation efficiency (FE), cell yield (CY) and gas production in ruminal fluid.

	Incubation Time (h)	Substrates						SEM	*p*-Value [1]		
		CS	GS	RS	SS1	SS2	SS3		SB	T	SB × T
FE (%)	8	84.98	81.88	79.83	82.89	83.60	84.10	0.243	<0.01	<0.05	0.20
	24	86.07	80.62	79.15	81.97	82.91	82.06				
CY [2]	8	2.09	2.60	2.26	1.82	1.86	1.86	0.07	<0.01	<0.01	0.60
	24	3.30	3.53	3.15	3.17	3.12	2.89				
Gas production [3]	8	38.63	54.43	37.32	29.87	32.00	27.34	0.327	<0.01	<0.01	0.11
	24	83.78	83.70	65.29	70.61	72.41	63.82				
Methane [3]	8	11.51	13.08	9.53	10.06	11.99	10.69	0.108	<0.01	<0.01	0.20
	24	25.49	23.30	16.64	22.33	23.60	18.60				

CS, corn silage; GS, grass silage; RS, rye silage; SS1, sorghum silage (sweet sorghum); SS2, sorghum silage (grain sorghum); SS3, sorghum silage (dual-purpose sorghum); SEM, standard error of the mean; [1] SB, effect of the substrate; T, effect of time; SB × T, interaction effect of substrate and time; [2] g/L of undiluted ruminal fluid; [3] mmol/L of undiluted ruminal fluid.

No interactions between substrates and fermentation time were noted in the results of our experiment (Tables 3 and 4), which indicates that the changes observed under the influence of the substrates were independent of the fermentation period, and thus, the effects might be more predictable.

4. Discussion

The most important products of the anaerobic microbial fermentation of carbohydrates in the alimentary tract of ruminants are volatile fatty acids, which cover 80% of the animals' demand for gross energy [32].

Acetic, propionic and butyric acids are the predominant volatile fatty acids in the rumen. They are easily absorbed into the bloodstream and transported to the cells, where they participate in gluconeogenesis, lipogenesis and synthesis of milk fat [33].

However, our study showed a lower P:B ratio after the addition of SS to the incubated digesta in comparison with rye or grass silage.

The results suggest no negative impact of the feedstuffs used in the study on the metabolic energy obtained in the process of ruminal fermentation. It is known that the energy in acetic, propionic and butyric acids is 62, 109 and 78% of that in hexose fermentation. Thus, the metabolically useful energy recovered in fermentation end products can be

increased by enhancing the production of propionic and, to a lesser extent, butyric acid at the expense of acetic acid production [29].

The lower total VFA concentration and the shift in VFA profile obtained in our research resulting from sorghum silage incubation may be due to the high NDF content in SS (Table 1), which usually leads to decreased VFA production with higher acetate and lower propionate proportions. A higher NDF to starch ratio included in the NSC ratio led to a decrease in VFA concentration and shifted the ruminal fermentation pattern towards higher acetic acid and lower propionic acid molar proportions [34]. The authors of an in vivo study of dairy heifers also observed an increase in the proportion of acetic acid and a decrease in propionic acid in the ruminal fluid after feeding with feedstuffs containing sorghum silage as roughage [13]. In our research, corn silage, because of its relatively high content of easily digestible NSC, caused a higher intensity of microbial fermentation in ruminal fluid than in SS samples, which resulted in a higher VFA concentration with decreased acetic and increased propionic and butyric acid molar proportions. These effects correlate with the study by Khaing et al. [35], which showed that increasing the proportion of corn silage in a goat diet resulted in increased molar proportions of propionic and butyric acids and a decreased proportion of acetic acid in the ruminal fluid.

Branched-chain VFAs in the rumen are the result of bacterial oxidative deamination and decarboxylation of valine, leucine and isoleucine. Previous research showed that the presence of these isoacids reflects the proteolytic activity of the ruminal microbiota [36]. The relatively low isobutyrate proportion in the SS samples in our study may indicate lower proteolytic activity for microbiomes affected by sorghum silage.

FE based on the volatile fatty acid profile illustrates the microbial activity in rumen digesta [29]. This value increased in our research probably as a result of the high NSC content in the fermentable substrates used, which was the highest in CS and lowest in RS (Table 1). Calabrò et al. [37] observed that in vitro fermentation of beef cattle ruminal fluid when sorghum silage was added as a fermentation substrate was less rapid than when corn silage was used. The results obtained in our study correspond to theirs, because VFA production, FE and microbial growth were lower in samples with sorghum silage than in those containing CS. Less rapid fermentation in the rumen results in stable pH for the ruminal fluid and reduces the risk of metabolic diseases, such as acidosis [29]. In our study, similar fermentation intensity of SS2 and other SS samples, despite relatively high NSC content, may depend on the content of slowly digestible and resistant starch [38], since most NSC in cereals is made up of starch.

Increasing the level of fermentable starch production in the rumen increases the VFA concentration in vivo [39]. In the authors' research, the VFA level in the SS group was lower than in the CS group, along with reduced NSC.

The type and quantity of structural (NDF and ADF) or nonstructural (NSC) carbohydrates in ruminant diet directly affect the rumen metabolism, lactic and VFAs concentrations and proportions, and the same amount of available energy. The NSCs consist mostly of starch, water-soluble carbohydrates, glucans, and pectins. The optimal content of NSC in milking cows' diets should range between 30–40% of DM [40]. The recommended dietary levels of NSC were found in presented study for corn (CS) and grain sorghum silages (SS2). Starch, which is the main component of NSC, is mainly fermented in the rumen to lactate and propionate but part of it is undegraded fraction in the rumen (by-pass starch) in further stage is broken down in the small intestine into glucose. The effective rumen degradability of starch depends on chemical composition of feedstuffs and animal diet. In the presented study, the highest value of propionate concentration in rumen fluid incubation despite the low NSC content in rye silage results from high effective rumen degradability of starch found for cereals. The lowest concentration of propionic acid (18.48 mol /100 mol) during fermentation of this substrate, despite the high content of NSC (33,93% of DM), indicates a lower effective rumen degradability of starch in grain sorghum silages (SS2) than in the rest of the substrates. In cows, reducing methanogenesis in the rumen is highly desirable, since methane production causes a loss of gross energy by the animals. Also, methane is

one of the greenhouse gases, and methanogenesis in the digestive tract of animals, especially ruminants, largely contributes to its emission. Manipulating their diet is a direct method of limiting their production of CH_4 [41,42].

Similar to our results, earlier research demonstrated that the use of sorghum silage, as compared to corn silage, in in vitro examination resulted in a reduced total production of fermentation gas in the rumen of beef cattle [37]. On the other hand, Campanili et al. [43] did not report any differences in gas production of ruminal fluid collected from beef steers fed diets containing corn silage or sorghum silage. Gas production depends, among other factors, on the NDF and ADF levels in feedstuffs, so a higher content of these fibre fractions will result in lower digestibility and lower gas production [44,45]. On the other hand, it was indicated that crude fibre produces more methane than starch [46], so it would be expected that greater methanogenesis would occur in SS than CS samples. Nevertheless, our results confirm the earlier in vivo research conducted on cows in metabolic cages: with feedstuffs containing sorghum silage, the absolute production of CH_4 was not higher than with those containing corn silage [47]. Similarly, ruminal fluid collected from beef steers fed diets containing corn silage or sorghum silage also showed unchanged amounts of methane [43]. However, recent studies indicated that the addition of suitable inoculants to sorghum ensiling, such as *Lactobacillus casei* TH14, can mitigate ruminal methane emission in animals [48]. Wilk et al. [49] obtained a positive effect with the addition of *Lactobacillus buchneri* on the ruminal degradability of ensiled sucrosorgo bagasse in vitro.

5. Conclusions

The obtained results of VFA profile and methane production indicate that dual-purpose, sweet and grain sorghum silages do not have a deleterious effect on microbial activity in the rumen. All sorghum silages used as substrates in ruminal fermentation cause a decrease in the total VFA concentration and the proportion of propionic acid in the ruminal fluid. The analysis of the study findings suggests that the addition of SS, irrespective of the plant variety, or RS will reduce fermentation gas production in the ruminal fluid as compared with CS. In conclusion, dual-purpose, sweet and grain sorghum silages may be used in feed for dairy cows as substitutes not only for corn but also for grass silage. Moreover, including rye silage in the feed ration reduces methanogenesis in the rumen. However, the final evaluation of the impact of the analysed silages on microbial fermentation in the rumen should be verified in in vivo studies.

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Institutional Review Board Statement: This study complied with the 3Rs, and all the procedures involving animals were in accordance with the ethical standards of the Wroclaw University of Environmental and Life Sciences, where the studies were conducted, as well as the Act of 15 January 2015 on the protection of animals used for scientific or educational purposes, implementing Directive 2010/63/EU on the protection of animals used for scientific purposes. Moreover, in compliance with Polish law, this study did not require the consent of the local ethics committee (request number 34/2017 from the Local Ethics Committee for Experiments on Animals in Wroclaw, Poland to E.P.-K.).

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