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EFFECT OF *SAPONARIA OFFICINALIS* L. OR *PANAX GINSENG* C.A MEYER TRITERPENOID SAPONINS ON RUMINAL FERMENTATION *IN VITRO**

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Abstract

This study examined the potential effects of *Saponaria officinalis* (SO) or *Panax ginseng* (PG) saponins supplemented to diets differing in the proportion of forage to concentrate on rumen microbial fermentation and methane production *in vitro*. Two experiments were carried out using the rumen simulation technique (RUSITEC). In the first experiment the substrate was comprised of a mixture of meadow hay and corn meal in a ratio of 60:40 dry matter (DM; high forage diet; HF). In the second experiment low forage diet (LF) consisting of meadow hay and corn meal in the ratio of 40:60 DM was used. Diets were supplemented with dried roots of SO or PG to provide 1% of triterpenoid saponins in dietary dry matter. All triterpenoid sources significantly (P≤0.05) decreased number of protozoa, by 50% and 72% respectively when HF diet was analysed (first experiment). There were no changes in methane production. In the second experiment (LF), the potential to mitigate methane production was reported for both SO and PG addition, however only in PG a decrease in the protozoal population was detected. Supplementation of plants rich in triterpenoid saponins has been identified as a diet dependent potential factor which has an important role in modulation of rumen fermentation processes. However, further studies are needed to evaluate their effect in animal production.

Key words: triterpenoid saponins, ruminal fermentation, volatile fatty acids, *in vitro*

Thanks to the microbial colonization of the digestive tract, primarily the rumen, ruminants may utilize low quality forages as the source of nutrients. However, oc-

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casionally even high quality forages in combination with concentrates cannot cover high nutrient requirements of high-producing animals. Many researchers are examining feed additives, which would facilitate enhanced utilization of nutrients supplied in the feed ration and thus reduce the adverse environmental impact of animal production. In recent years, e.g. in view of the ban on the application of antibiotic growth promoters in animal nutrition (Regulation (EC) No 1831/2003), bioactive phytofactors started to be intensively investigated as the potential antibiotic growth promoter substitutes. Highly diverse bioactive components contained in many plant species have been identified as potential agents capable of modulating rumen fermentation processes. In animal nutrition phytofactors may be supplied as the plant dietary ingredients (e.g. dried leaves) or extracts containing pure forms of phytofactors. Triterpenoid saponins are examples of bioactive phytochemicals, whose action is closely related with their structure. These high molecular glycosides are composed of hydrophobic sapogenin and a hydrophilic saccharide radical glycone, which in the case of triterpenoid saponins is a triterpene (Sparg et al., 2004). Saponins are commonly found in many plants and plant origin products and because of their antimicrobial potential may play important roles in animal and human nutrition (Makkar et al., 2007; Nasri et al., 2011). Triterpenoid saponins may affect cell membrane permeability and thus exhibit antimicrobial action (Francis et al., 2002). In the digestive tract of ruminants, primarily in the rumen, they may have antibacterial, antiprotozoan, antifungal and antimethanogenic effects (Hu et al., 2005 a; Agarwal et al., 2006; Goel et al., 2008). Thanks to the multifaceted action of bioactive phytochemicals they may affect rumen processes, including protein metabolism or methanogenesis, thus reducing environmental pollution by ammonia and methane (Szumacher-Strabel and Cieślak, 2012; Cieślak et al., 2012). Due to the abundance of bioactive phytochemicals found in nature, continuous research is being conducted in order to optimize the concentration and form, in which a given bioactive phytochemical should be introduced to feed rations for ruminants (Zmora et al., 2012). Despite many studies conducted so far on the use of saponins, in terms of the selective modulation of rumen microbial population for limitations of methanogenesis or proteolysis in the rumen ecosystem, the plant source of saponins that would fully meet the expectations has not been found yet (Szumacher-Strabel and Cieślak, 2010). In the case of saponin application most literature sources report the use of *Yucca schidigera* as a source of steroid saponin, while a limited number of studies were conducted with triterpenoid saponins. Moreover, a previous study demonstrated that effects of supplemented feed additives on quantitative as well as qualitative variability of microorganisms in the rumen depends on many factors, such as type of feed ration with particular reference to the composition and proportions of individual nutrients (Cieślak et al., 2006; Szumacher-Strabel and Cieślak, 2010). Therefore the objective of this study was to assess the effects of *Saponaria officinalis* L. (SO) or *Panax ginseng* C.A Meyer (PG) saponins supplemented to high forage (HF) or low forage (LF) diets on rumen microbial fermentation and methane production *in vitro*.

Material and methods

Materials

As the sources of triterpenoid saponins dried roots of *Saponaria officinalis* L. (SO) or *Panax ginseng* C.A Meyer (PG) were used. Plant material was purchased from Herbapol (Kraków, Poland). The voucher samples have been deposited at the Department of Animal Nutrition and Feed Management, Poznan University of Life Sciences. For each saponin source total triterpenoid saponins content was determined.

Experimental design

Two *in vitro* experiments were conducted. In the first experiment high forage diet (HF) consisted of meadow hay and corn meal in a ratio of 60:40 DM, whereas in the second experiment low forage diet (LF) consisting of meadow hay and corn meal in a ratio of 40:60 was used (Table 1). Both experimental diets (HF and LF) were supplemented with dried roots of SO or PG to provide 1% of triterpenoid saponins of dietary dry matter, 3.8 g/day or 2.6 g/day, respectively.

Diet preparation

Samples of feeds were analysed according to AOAC (2007, Table 1) for dry matter (method no. 934.01) and ash (method no. 942.05). Crude protein was determined by Kjel-Foss Automatic 16210 analyser (method no. 976.05), crude fat by Soxtec System HT analyser (method no. 2003.05), and neutral detergent fibre (NDF) (with amylase treatment) was determined according to the methods of Van Soest et al. (1991).

Table 1. Chemical composition of feeds used in the study, n = 3

Items	Dry matter (g/kg)	Crude protein (g/kg DM)	Ash (g/kg DM)	Crude fat (g/kg DM)	NDF[1] (g/kg DM)	NFC[2] (g/kg DM)
Meadow hay	917.57	118.23	49.87	15.33	565.70	250.87
SEM[3]	6.64	9.42	7.38	1.90	1.10	3.28
Corn meal	870.73	107.23	14.10	40.63	86.20	751.83
SEM	17.98	2.92	0.23	2.11	0.35	4.20

[1]NDF – neutral detergent fibre.
[2]NFC – non-fibrous carbohydrates.
[3]SEM – standard error of the mean.

Extraction and isolation of saponin fraction from *Saponaria officinalis* and *Panax ginseng*

Extraction and isolation of saponin fraction from *Saponaria officinalis* and *Panax ginseng* were performed according to the procedure described by Hamed et al. (2012). Briefly, samples of plant material (100 mg) were mixed with diatomaceous earth and extracted with 80% methanol in stainless steel extraction cells of an accelerated solvent extraction system (ASE 200, Dionex, Sunnyvale, USA). Extractions were carried out at 10 MPa operating pressure and temperature of 40°C.

After evaporation to dryness under reduced pressure, extracts were redissolved in 2 mL of distilled water and applied at the top of a Waters Sep-Pak Classic 360 mg SPE column (Waters, Milford, USA) activated with 2.5 mL of methanol and 2.5 mL of distilled water. After a 5 mL wash with 40% methanol (to remove sugars and phenolic compounds), the compounds of interest were eluted with 3 mL of 100% methanol. Samples were then dried *in vacuo* (vacuum 28 mbar, rotation 100 rpm, temperature 40ºC), redissolved in 50% methanol and analysed by LC-MS (Liquid Chromatography–Mass Spectrometry).

Determination of saponins in the root of *Panax ginseng*

Quantitative analyses were performed on an Acquity Ultra Performance Liquid Chromatography (UPLC) system equipped with a triple quadruple mass spectrometer (Waters, Milford, USA). Ginsenosides were separated on a Waters BEH (Bridged Ethylene Hybrid) C18 column (100 × 1 mm, 1.7 µm) with a linear (12 min) gradient from 25 to 45% of acetonitrile containing 0.1% (v/v) formic acid (solvent B) in 0.1% formic acid (solvent A) and flow rate of 140 µl/min. The separation was carried out at column temperature of 50°C. One µl sample was injected in the "partial loop needle overfill" mode of a Waters Acquity autosampler.

Column's effluent was introduced into the ion source of the mass spectrometer, which operated in the negative ion mode with the following parameters of the ion source: cone voltage 130 V, capillary voltage 3.2 kV, extractor 3 V, RF lens 100 mV, source temperature 130°C, desolvation temperature 350°C, desolvation gas flow 700 l/h, cone gas flow 50 l/h. Collision cell entrance and exit were set to 50, collision energy was set to 5. Parameters of quadruples 1 and 3 were set to achieve unit-mass resolution: both LM and HM resolutions were set to 15, and ion energies were set to 0.9.

It was found that the dried root of PG contained 42.54 mg/g saponins as equivalent of ginsenoside Re {2-O-(6-deoxy-α-L-mannopyranosyl)-(3β,6α,12β)-20-(β-D-glucopyranosyloxy)-3,12-dihydroxydammar-24-en-6-yl-β-D-glucopyranoside}.

Determination of saponins in the root of *Saponaria officinalis*

Quantitative analyses of extracts were done using a Thermo LCQ Advantage Max ion-trap mass spectrometer coupled with a Surveyor high-performance liquid chromatography (HPLC) system composed of a Surveyor PDA, an autosampler and a quaternary pump (Thermo, Waltham, USA). Separation was performed on a 150 × 2.1 mm i.d., 3.5 µm Symmetry C18 column (Waters, Milford, USA) using a linear, 90 min gradient from 5 to 60% of solvent B (acetonitrile containing 0.03% formic acid) in solvent A (doubly distilled water containing 0.03% formic acid) with a flow of 0.4 mL/min. The column was held at 50°C during separations and re-equilibration. The mass spectrometer was operated in the negative electrospray mode with the following ion source parameters: spray voltage 3.5 kV, capillary voltage –47 V, tube lens offset –60 V, transfer capillary temperature 230°C. Nitrogen sheath and auxiliary gas flows were, respectively, 65 and 10 arbitrary units. The acquisition mode was data-dependent. Following a general scan from 150 to 2000 mass units (3 microscans, max. inject time of 150 ms), peaks with signals higher than 2.5×10^5

counts were subjected to MS/MS analysis. In each scan, the peak with the highest intensity was fragmented using collision-induced dissociation (CID, 35% normalized collision energy) and product ions in the range from the "cutoff" to the parent mass were detected. MS/MS fragmentation data were used only to tentatively identify the compounds having saponin nature. Quantitations were based on UV absorbance at 210 nm wavelength.

It was shown that dried root of SO contained 30 individual saponins in a total amount of 28.76 mg/g of dry matter as equivalent of saponarioside I {3-O-*β*-D--xylopyranosyl-16*α*-hydroxygypsogenic acid 28-O-*α*-D-galactopyranosyl-(1→6)-*β*-D- glucopyranosyl-(1→3)[- *β* -D-glucopyranoside}.

Equipment and technique

The study was carried out using RUSITEC apparatus (Czerkawski and Breckenridge, 1977) consisting of four vessels with an effective volume of 1 l each, immersed in a water bath maintained at 39°C. Fermentation inoculum (rumen fluid and solid digesta) for starting *in vitro* experiments were collected from three rumen cannulated Polish Holstein-Friesian dairy cows (body weight 600±25 kg) fed 20 kg/day of DM. The dairy cow's diet was composed of alfalfa silage (10.4 kg DM), meadow hay (1.6 kg DM), wet brewers' grains (0.5 kg DM), soybean meal (1.4 kg DM) and commercial concentrate containing 19% of crude protein (6.1 kg DM). Drinking water was available *ad libitum*. The solids rumen digesta and rumen fluid were collected before morning feeding. Fermentation inoculums from each cow were combined and thoroughly mixed, then transported to the laboratory within an hour in pre-heated vacuum flasks and transferred to a water bath with continual CO_2 purging. On the first day of each experimental run, each of the 1 l fermenters from Rusitec system, was initially filled with pre-warmed 100 ml of McDougall buffer (McDougall, 1948) and 820 ml of ruminal fluid, that was earlier strained through 4 layers of cheesecloth. Moreover, two nylon bags (70×140 mm, 100-µm pore size) for one fermenter were used: one containing ca. 11 g DM of rumen digesta solids and the second containing an experimental diet (10.9 g of DM). After 24 h of incubation, the system was opened and the bag containing rumen digesta solids was replaced by another bag containing the experimental diet. Anaerobic conditions were re-established in the fermenters by rinsing the system with gaseous N_2 for 3 min (3 litres/min) after the daily supply of substrate was completed. Artificial saliva was supplied to each fermenter at a flow rate of 500 ml/day using a peristaltic pump. Displaced effluent and fermentation gases were collected in an effluent vessel and gas-tight collection bag (TECOBAG 81; Tesseraux Container GmbH, Bürstadt, Germany), respectively. To stop fermentation in the effluent vessels, 10 ml of 6N HCl was added to each of them.

Measurements and chemical analysis

Three experimental runs of 10 days each were carried out; the first 5 days served as an adaptation period followed by 5-day measurements. The samples of rumen fluid fermentation parameters were collected 3 h before the addition of the new bag with feed. The pH was measured immediately after collection, using a pH meter type CP-104. The ammonia concentration was analysed by the colorimetric Nessler

method as modified by Szumacher-Strabel et al. (2002). The protozoa counts were determined according to Michalowski et al. (1986) using a drop of buffered rumen fluid with defined volume (100 µl) under a light microscope (Zeiss, type Primo Star no. 5, Jena, Germany). The total bacteria population was obtained with a Thoma counting chamber (Blau Brand®, Wertheim, Germany) (Ericsson et al., 2000). For the determination of volatile fatty acids (VFA), 3.6 ml of the buffered fluid sample was stabilized with 0.4 ml of a 46 mM $HgCl_2$ solution and frozen (−20ºC) until analysis by GC (GC Varian CP 3380, Sugarland, TX, USA), in accordance with the guidelines, described by Tangerman and Nagengast (1996). The content of non-fibre carbohydrates (NFC) was calculated according to the following equation: *1000 – (crude protein + crude fat + ash + NDF*, and expressed as g/kg DM (Cozzi et al., 2002).

In vitro organic matter digestibility (IVOMD, %) was estimated according to Menke et al. (1979) as follows:

$$IVOMD\ (\%) = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ XA$$

where:

CP – crude protein expressed in percent of DM,

XA – ash expressed in percent of DM,

GP – the net gas production expressed in ml from 200 mg of DM after 24 h of incubation.

The volume of net gas production was obtained by pressing the gas into a closed tube filled with water and measuring the amount of displaced water.

Methane production was calculated based on the theoretical fermentation balance for the measured molar proportion of VFA and OM fermented in the rumen according to Wolin (1960) as follows: 57.5 mol glucose = 65 mol acetate + 20 mol propionate + 15 mol butyrate + 60 mol CO_2 + 35 mol CH_4 + 25 mol H_2O.

Statistical analysis

Differences between diets composition were tested with t-test, and due to statistical differences between HF and LF diets, effects of SO and PG diet supplementation were analysed separately for each experiment (HF and LF). General linear model (GLM) procedure of SAS (2006) was used for data analysis, assuming the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + E_{ijk}$$

where:

Y_{ijk} – value of the observation,

μ – mean,

C_i – effect of the sample,

P_j – effect of the day,

T_k – effect of the diet supplementation (SO, PG and control),

E_{ijk} – random error.

$P<0.05$ was considered as being statistically significant. Differences between means were tested using Duncan test.

Results

The content of triterpenoid saponin was 28.76 mg/g of dry matter (DM) in SO roots and 42.54 mg/g DM in PG roots. The applied *in vitro* doses (HF or LF) differed significantly in the level of neutral detergent fibre and non-fibrous carbohydrates (Table 2). In the first experiment, where HF was used, pH value was decreased (P≤0.05) in experimental groups compared with the control (Table 3). Addition of SO increased (P≤0.05) the IVOMD, whereas the PG had no effect on the measured parameter. The particular and total VFA have been altered in the presence of triterpenoid saponin. The PG and SO saponins significantly changed the VFA profile by increasing the acetate and propionate contents in comparison with the control (Table 3). Thus, the ratio of acetate to propionate has been decreased: by 18% when the saponins derived from SO and by 51% when saponins from PG were supplemented. Addition of PG increased (P≤0.05) butyrate, iso-butyrate and iso-valerate concentrations. The dietary supplementation of SO and PG decreased (P≤0.05) the number of protozoa by 50% and 72%, respectively (Table 3).

Table 2. The chemical composition of HF or LF doses

Items	HF[1]	SEM[5]	LF[2]	SEM
Dry matter (g/kg)	10.80	0.120	10.69	0.145
Crude protein (g/kg DM)	1.24	0.051	1.22	0.027
Organic matter (g/kg DM)	10.41	0.163	10.39	0.172
Crude fat (g/kg DM)	0.28	0.007	0.33	0.008
NDF[3] (g/kg DM)	4.08 A	0.006	3.03 B	0.003
NFC[4] (g/kg DM)	4.92 B	0.003	6.01 A	0.013

[1]HF – high forage diet.
[2]LF – low forage diet.
[3]NDF – neutral detergent fibre.
[4]NFC – non-fibrous carbohydrates.
[5]SEM – standard error of the mean.
A, B – means with different superscripts in each row are significantly different (P<0.05).

In the second experiment, where LF was used, PG supplementation significantly decreased (P≤0.05) the pH value (Table 4). The dietary supplementation of SO significantly decreased (P≤0.05) ammonia concentration in comparison to LF diet supplemented with PG (Table 4). Similarly, as in the first experiment, SO addition increased (P≤0.05) the IVOMD, whereas PG had no effect on this parameter. Dietary supplementation of PG and SO resulted in 8% lower CH_4 concentrations than in the control group. Total VFA concentration was significantly higher (P≤0.05) when PG was supplied compared to the control or SO diets. Supplementation with PG also increased the molar concentrations of propionate, butyrate, iso-butyrate and iso-valerate. VFA profile of rumen fluid incubated with PG lowered the acetate and valerate contents and the acetate to propionate ratio, by 22%, 57% and 45% respectively, when compared to the controls (Table 4). Only triterpenoid saponin from PG significantly decreased (P≤0.05) total protozoa population in comparison to the control, whereas SO had no effect on the analysed population (Table 4).

Table 3. Effects of triterpenoid saponins of *Saponaria officinalis* (SO) or *Panax ginseng* (PG) supplementation to HF diet on fermentation parameters and microbial populations, n = 15

	Control		SO		PG	
	Mean	SEM[1]	Mean	SEM	Mean	SEM
Fermentation parameters						
ruminal pH	7.06 A	0.01	6.99 B	0.01	6.80 C	0.01
ammonia (mmol/l)	7.92	1.08	7.34	0.29	10.53	0.39
IVOMD[2] (%)	54.08 B	1.58	59.01A	2.99	53.38 B	2.05
methane (mM)	0.28	0.02	0.29	0.01	0.27	0.02
total VFA[3] (mmol/l)	52.09 C	0.55	59.3 B	0.64	80.51 A	1.90
VFA concentration (mmol/l)						
acetate	28.34 B	0.22	31.29 A	0.32	30.09 A	0.41
propionate	8.94 C	0.12	12.09 B	0.23	19.50 A	0.80
butyrate	1.95 B	0.07	2.48 B	0.18	3.31 A	0.15
iso-butyrate	8.19 B	0.22	8.10 B	0.25	19.19 A	0.34
valerate	2.36 AB	0.06	3.00 A	0.18	1.22 B	0.13
iso-valerate	2.31 B	0.19	2.34 B	0.15	7.20 A	0.32
ratio of acetate to propionate	3.18 A	0.06	2.60 B	0.06	1.56 C	0.05
Microbial population						
protozoa counts ($\times 10^3$ cells/ml)	3.44 A	0.57	1.72 B	0.44	0.95 B	0.00
bacteria counts ($\times 10^8$ cells/ml)	72.00	15.15	106.00	18.70	113.00	10.17

[1]SEM – standard error of the mean.
[2]IVOMD – *in vitro* organic matter digestibility.
[3]VFA – volatile fatty acids.
A, B, C – means with different superscripts in each row are significantly different (P<0.05).

Table 4. Effects of triterpenoid saponins of *Saponaria officinalis* (SO) or *Panax ginseng* (PG) supplementation to LF diet on fermentation parameters and microbial populations, n = 15

	Control		SO		PG	
	Mean	SEM[1]	Mean	SEM	Mean	SEM
Fermentation parameters						
ruminal pH	6.97 A	0.01	6.98 A	0.01	6.86 B	0.01
ammonia (mmol/l)	4.58 AB	0.77	2.63 B	0.15	6.62 A	0.57
IVOMD[2] (%)	45.22 B	2.04	49.65 A	1.61	42.61 B	1.15
methane (mM)	0.25 A	0.01	0.23 B	0.01	0.23 B	0.01
total VFA[3] (mmol/l)	52.41 B	1.39	49.11 B	1.10	60.97 A	0.30
VFA concentration (mmol/l)						
acetate	24.82 A	0.53	23.65 A	0.49	19.26 B	0.35
propionate	9.38 B	0.27	9.05 B	0.22	13.22 A	0.20
bytyrate	3.62 B	0.13	3.62 B	0.09	4.48 A	0.05
iso-butyrate	8.70 B	0.28	7.77 B	0.21	13.96 A	0.09
valerate	2.85 A	0.10	2.25 A	0.07	1.21 B	0.18
iso-valerate	3.05 B	0.11	2.77 B	0.08	8.84 A	0.10
ratio of acetate to propionate	2.66 A	0.03	2.62 A	0.02	1.46 B	0.04
Microbial population						
protozoa counts ($\times 10^3$ cells/ml)	2.29 A	0.14	2.29 A	0.22	0.96 B	0.00
bacteria counts ($\times 10^8$ cells/ml)	68.00	6.27	86.80	12.52	74.80	5.91

[1]SEM – standard error of the mean.
[2]IVOMD – *in vitro* organic matter digestibility.
[3]VFA – volatile fatty acids.
A, B – means with different superscripts in each row are significantly different (P<0.05).

Discussion

The supplementation of ruminant diets with feed additives containing saponins can reduce the processes of protein degradation and mitigate the methanogenesis in the rumen ecosystem. These can improve nutrient utilization of feed by the animals. In the current study, addition of SO or PG to the experimental diets, only in the case of LF diet, decreased methane production. This suggested that not only saponin sources but also type of the diet fed (HF vs LF) may affect the results obtained.

Moreover, in the present study pH value of the rumen fluid was reduced by applied additives ($P \leq 0.05$), except for SO in LF diet, but still it was maintained in the physiological range. Hu et al. (2005 b) in experiments conducted under *in vitro* conditions stated the effect of tea triterpenoid saponin addition on a reduction of pH value in incubated ruminal fluid. A decrease in pH may be caused by an increase in the concentration of total volatile fatty acids, which was also observed in the presented study. An addition of PG, irrespective of the applied feed ration, resulted in a statistically significant ($P \leq 0.05$) increase in total VFA. In turn, in the case of SO a statistically significant increase was found in total VFA in the feed ration with HF. As VFA constitute a primary source of energy for ruminants, the results recorded within this study are advantageous from the production practice point of view. An increased total VFA concentration was also reported by other researchers in their experiments conducted with an addition of steroid saponins (Lila et al., 2003). The direct cause of an increase in total VFA is connected with an increase in the concentrations of individual fatty acids, including acetate and propionate. In the conducted experiments the content of acetate, under the influence of applied experimental factors, increased in the case of the HF ration, while supplementation of LF diet with PG decreased acetate concentration. Hence, the decrease in acetate to propionate ratio ($P \leq 0.05$) was found in the case of the application of PG in both analysed diets and in HF diet when SO was supplemented.

The propionate concentration was higher ($P \leq 0.05$) for HF diets when SO and PG were supplemented and also in the LF diets when PG was added. A higher propionate concentration may be associated with reduction of protozoa population, which was observed in the current study. Moreover, the average concentration of butyrate, isobutyrate and iso-valerate was considerably higher ($P \leq 0.05$) for PG compared with SO, regardless of the type of diet fed. Based on the conducted experiments it may be stated that the effect of applied additives, triterpenoid saponin carriers, depended not only on the type of applied feed ration, but also on the saponin carrier, and PG supplement seems to be more effective in increasing both the total amount and particular concentration of VFA in tested conditions. Higher IVOMD was reported for SO supplements in both diets, which was the opposite of the PG supplements. These results are consistent with data presented by Patra and Saxena (2009), who also showed that the effect of phytofactors depends on their origin, the diet composition and adaptation of microbiota to rumen environment.

The composition of the feed ration had a decisive effect on CH_4 production. Forages, in which NDF is found in greater amounts, are more methanogenic than concentrates. Based on the results obtained it may also be concluded that the sup-

plementation of LF diet with SO or PG caused a statistically significant reduction of methane production. In the case of SO, the decreased methane production with no limitation of the protozoa and bacteria populations suggested that saponins contained in this additive must have had a direct effect on the population size or activity of methanogens. In turn, a lack of reduction of methane production in the HF ration may be explained by the tendency to increase of bacterial counts, which as a result of their metabolism lead to the generation of considerable amounts of hydrogen, a substrate for the production of methane.

According to Wallace (2004) saponins reduce the level of ammonia in the rumen fluid. Also investigations conducted *in vivo* by Mao et al. (2010) indicated that an addition of tea saponins reduced ammonia content in the rumen fluid. However, such a dependence was not confirmed in this study. Average ammonia concentration reported for PG was higher than for SO in both diets, however the differences were statistically significant only in LF diet. It is difficult to interpret our results relative to available literature. The application of bioactive phytochemicals (e.g. essential oils) typically results in the reduced amount of ammonia in the rumen as a consequence of a decrease in deamination and peptidolysis. Moreover, it was found that the use of these compounds may result in decreased number and activity of bacteria specialized in ammonia production (referred to as hyper ammonia producing bacteria), thus leading to the limitation of the production of excess ammonia in the rumen (Szumacher-Strabel and Cieślak, 2010). In the case of SO and PG application, the recorded results might be influenced by the duration of the effect or the applied concentration of saponins. Moreover, in the analysis of conducted experiments we need to consider other bioactive substances (e.g. carbohydrate fractions or phenolic acids), which, when interacting with saponins or components of the feed ration, may yield in completely different results than in the case of pure forms being used.

Bacteria found in the rumen are responsible for many metabolic changes, including also those of nitrogen. However, a varied action of saponins on different bacterial groups was shown (Wang et al., 2000). Cellulolytic bacteria are more resistant to the action of saponins than amylolytic bacteria, which was also indirectly observed in the conducted experiments (a higher numerical bacterial count in the HF ration). However, earlier studies showed that the addition of a yucca extract caused a reduction of population size in the case of bacteria commonly found in the digestive tract of ruminants, i.e. *Butyrivibrio fibrisolvens* (decomposing cellulose) and *Streptococcus bovis* (decomposing urea), but they had a positive effect on the count of *Prevotella ruminicola* (decomposing starch), whereas no effect was observed on ammonia-producing *Selenomonas ruminantium* (Wallace et al., 1994). This indicated that the data obtained for the bacterial populations are not always consistent. As regards the effect of saponin on protozoa population, this value is considered to be one of the most effective factors leading to a reduction of population size in this group of microorganisms, which was also confirmed in experiments with SO and PG in HF diet and PG in LF diet. However, more effective effect on the protozoa population (a reduction by as much as 72%) was found for the HF diet. This may suggest that the HF diet could have contained a lower amount of protein, which may not have entered into interactions with saponins contained in both SO and PG; thus,

this would not have blocked the reduction of the protozoan population size under the influence of the applied experimental factors. However, this hypothesis, in the case of the conducted experiments is not confirmed due to the fact that protein content in the two tested diets was similar. Makkar et al. (2007), Wallace et al. (2002) and Eryavuz and Dehority (2004) stated that the generally negative effect of saponins on protozoa cells is caused by a reduced surface tension in the cell membranes of protozoan through the action of saponins, leading to their decreased permeability. In the case of bacteria, a lack of the toxic effect of saponins is explained by the absence of sterols in the cell membrane and thus a lack of the negative effect on permeability of bacterial cell membranes (Williams and Coleman, 1992). This theory was indirectly confirmed by investigations conducted within the presented experiments with an addition of SO and PG.

In conclusion, the addition of triterpenoid saponins, regardless of the source of their origin (SO or PG), resulted in changes in the rumen fluid parameters *in vitro*. Moreover, the additives, regardless of the forage to concentrate ratio (resulting in different content of the neutral detergent fibre and non-fibrous carbohydrates), showed a significant role in regulating the number of protozoa in the rumen fluid after incubation, and therefore the additives may be used as effective defaunation agents. Based on the results obtained it may also be concluded that addition of triterpenoid saponins from SO or PG in combination with a low forage diet have greater potential to reduce methane production. However, further research is required to better understand the mechanism of ruminal fermentation and the effect of saponins on the process of methanogenesis.

References

Agarwal N., Kamra D.N., Chaudhary L.C., Patra A.K. (2006). Effect of *Sapindus mukorossi* extracts on *in vitro* methanogenesis and fermentation characteristics in buffalo rumen liquor. J. Appl. Anim. Res., 30: 1–4.

AOAC (2007). Association of Official Analytical Chemists. Official Methods of Analysis. 18th Edition. Gaithersburg Maryland, USA, Editors W. Horwitz and W. Latimer.

Cieślak A., Zmora P., Pers-Kamczyc E., Szumacher-Strabel M. (2012). Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. Anim. Feed Sci. Technol., 176: 102–106.

Cieślak A., Szumacher-Strabel M., Szymankiewicz E., Piękniewski M., Oleszak P., Siwiński Ł., Potkański A. (2006). Coconut oil reduces protozoa count and methane release during fermentation in a RUSITEC system. J. Anim. Feed Sci., 15: Suppl. 1: 19–22.

Cozzi G., Burato G.M., Berzaghi P., Andrighetto I. (2002). Evaluation of pellets from different industrial processing of dehydrated lucerne in dairy cattle feeding. Anim. Feed Sci. Technol., 99: 13−24.

Czerkawski J.W., Breckenridge G. (1977). Design and development of a long-term rumen simulation technique (Rusitec). Br. J. Nutr., 38: 371–384.

Ericsson M., Hanstorp D., Hagberg P., Enger J., Nystrom T. (2000). Sorting out bacterial viability with optical tweezers. J. Bacteriol., 182: 5551–5555.

Eryavuz A., Dehority B.A. (2004). Effect of *Yucca schidigera* extract on the microorganisms in sheep. Anim. Feed Sci. Technol., 117: 215–222.

Francis G., Kerem Z., Makkar H.P.S., Becker K. (2002). The biological action of saponins in animal systems: reviews. Br. J. Nutr., 88: 587–605.

Goel G., Makkar H.P.S., Becker K. (2008). Effects of *Sesbania sesban* and *Carduus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage- and concentrate-based feeds to methane. Anim. Feed Sci. Technol., 147: 72–89.

Hamed A., Janda B., Mahalel U., Stochmal A., Oleszek W. (2012). Profiles of steroidal saponins from the aerial parts of *Tribulus pentandrus*, *T. megistopterus subsp. pterocarpus* and *T. parvispinus* by LC-ESI-MS/MS. Phytochem. Anal., 23 (6): 613–621.

Hu W-L., Liu J-X., Ye J-A., Wu Y-M., Guo Y-Q. (2005 a). Effect of tea saponin on rumen fermentation *in vitro*. Anim. Feed Sci. Technol., 120: 333–339.

Hu W-L., Wu Y-M., Liu J-X., Guo Y-Q., Ye J-A. (2005 b). Tea saponins affect *in vitro* fermentation and methanogenesis in faunated and defaunated rumen fluid. J. Zhejiang Univ. Sci., 8: 787–792.

Lila Z.A., Mohammed N., Kanda S., Kamada T., Itabashi H. (2003). Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro*. J. Dairy Sci., 86: 3330–3336.

Makkar H.P.S., Francis G., Becker K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal, 1: 1371–1391.

Mao H.L., Wang J.K., Zhou Y.Y., Liu J.X. (2010). Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. Livest. Sci., 129: 56–62.

McDougall E.I. (1948). Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J., 43: 99–109.

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. Camb., 92: 217–222.

Michalowski T., Harmeyer H., Breves G. (1986). The passage of protozoa from the reticulo-rumen through the omasum of sheep. Br. J. Nutr., 65: 625–634.

Nasri S., Ben Salem H., Vasta V., Abidi S., Makkar H.P.S., Priolo A. (2011). Effect of increasing levels of *Quillaja saponaria* on digestion, growth and meat quality of Barbarine lamb. Anim. Feed Sci. Technol., 164: 71–78.

Patra A.K., Saxena J. (2009). The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr. Res. Rev., 22: 204–219.

SAS ® (2006) SAS/STAD User's Guide. SAS Institute Inc. Cary, NC.

Sparg S.G, Ligot M.E, van Staden J. (2004). Biological activities and distribution of plant saponins. J. Ethnopharmacol., 94: 219–243.

Szumacher-Strabel M., Cieślak A. (2012). Dietary possibilities to mitigate rumen methane and ammonia production, greenhouse gases – capturing, utilization and reduction, ISBN: 978-953-51-0192-5, InTech, Available from: http://www.intechopen.com/books/greenhouse-gases-capturing-utilization-and-reduction/dietary-possibilities-to-mitigate-rumen-methane-and-ammonia-production.

Szumacher-Strabel M., Cieślak A. (2010). Potential of phytofactors to mitigate rumen ammonia and methane production. J. Anim. Feed Sci., 19 (3): 319–337.

Szumacher-Strabel M., Potkański A., Kowalczyk J., Cieślak A., Czauderna M., Gubała A., Jędroszkowiak P. (2002). The influence of supplemental fat on rumen volatile fatty acid profile, ammonia and pH level in sheep fed standard diet. J. Anim. Feed Sci., 11: 577–587.

Tangerman A., Nagengast F.M. (1996). A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. Anal. Biochem., 236: 1–8.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583–3597.

Wallace R.J. (2004). Antimicrobial properties of plant secondary metabolites. Proc. Nutr. Soc., 63: 621–629.

Wallace R.J., McEwan N.R., McIntosh F.M., Teferedegne B., Newbold C.J. (2002). Natural products as manipulators of rumen fermentation. Asian-Australas. J. Anim. Sci., 15: 1458–1468.

Wallace R.J., Arthaud L., Newbold C.J. (1994). Influence of *Yucca-schidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol., 60: 1762–1767.

Wang Y.X., McAllister T.A., Yanke L.J., Xu Z.J., Cheeke P.R., Cheng K.J. (2000). *In vitro* effects of steroidal saponins from *Yucca schidigera* extract on rumen microbial protein synthesis and ruminal fermentation. J. Sci. Food Agric., 80: 2114–2122.

Williams A.G., Coleman G.S. (1992). Editors. The Rumen Protozoa. Springer-Verlag, New York, USA.

Wolin M.J. (1960). A theoretical rumen fermentation balance. J. Dairy Sci., 43: 1452.

Zmora P., Cieślak A., Jedrejek D., Stochmal A., Pers-Kamczyc E., Oleszek W., Nowak A., Szczechowiak J., Lechniak D., Szumacher-Strabel M. (2012). Preliminary *in vitro* study on the effect of xanthohumol on rumen methanogenesis. Arch. Anim. Nutr., 66 (1): 66–71.

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Wpływ saponin triterpenowych *Saponaria officinalis* L. lub *Panax ginseng* C.A. Meyer na przemiany zachodzące w żwaczu w warunkach *in vitro*

STRESZCZENIE

Celem przeprowadzonych badań było określenie wpływu ekstraktu saponin triterpenowych, *Saponaria officinalis* (SO) lub *Panax ginseng* (PG), w dawkach różniących się poziomem i rodzajem węglowodanów, na proces fermentacji w żwaczu i produkcję metanu w warunkach *in vitro*. Przeprowadzono dwa doświadczenia, wykorzystując dynamiczny system symulujący pracę żwacza (RUSITEC). W pierwszym doświadczeniu zastosowana dawka podstawowa składała się z siana łąkowego i śruty kukurydzianej w stosunku 60:40 suchej masy (dawka z wysokim udziałem pasz objętościowych – HF). W drugim eksperymencie przeanalizowano dawkę pokarmową z niskim udziałem pasz objętościowych (LF; siano łąkowe i śruta kukurydziana w stosunku 40:60 suchej masy). Do dawek podstawowych dodano saponiny triterpenowe, które stanowiły 1% suchej masy dawki. W pierwszym doświadczeniu (HF), zarówno SO, jak i PG znacząco ($P \leq 0,05$) ograniczyły liczebność pierwotniaków, odpowiednio o 50% i 72%. Nie odnotowano natomiast zmian w poziomie koncentracji metanu. W drugim doświadczeniu (LF) potencjał do ograniczania produkcji metanu wykazał zarówno dodatek SO, jak i PG, jednak tylko w przypadku PG zaobserwowano zmniejszenie liczby pierwotniaków. Na podstawie przeprowadzonych badań stwierdzono, iż rośliny bogate w saponiny triterpenowe odgrywają istotną rolę w regulacji procesów fermentacji w żwaczu. Jednak aby ocenić ich wpływ na produkcję zwierzęcą, konieczne jest przeprowadzenie dalszych badań.