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EFFECT OF SAPONIN FROM *BIOPHYTUM PETERSIANUM* KLOTZSCH EXTRACT ON RUMINAL FERMENTATION CHARACTERISTICS IN GOATS

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ABSTRACT

Four Kacang goats with an initial body weight (BW) of 20.25 ± 2.78 kg were used in a 4 × 4 Latin square design to determine effect of *Biophytum petersianum* Klotzsch saponin on ruminal pH, ammonia-N and volatile fatty acids (VFA) patterns, and protozoa number. The goats were fed twice daily (08.00 and 16.00 h) with a basal diet consisting of elephant grass silage and a concentrate (70:30 on a dry matter basis). Dietary treatments were basal diet (A), basal diet + 13 mg of saponin/kg BW (B), basal diet + 19 mg of saponin/kg BW (C), basal diet + 26 mg of saponin/kg BW (D). *Biophytum* extract was administered orally in amounts of 0, 160, 239 and 319 ml daily, corresponding to 0, 13, 19.5 and 26 mg of saponin/kg BW. Results showed that goats fed B, C and D diets had lower ($P<0.05$) rumen ammonia-N concentrations compared to the A diet. The pH values ranged from 6.91 to 7.06 and were higher ($P<0.05$) in sheep fed supplemented diets (B, C, and D) versus those fed control diet (A). Total VFA concentration, and molar proportions of butyrate, valerate and iso-acids were similar ($P>0.05$) among saponin treatments. Protozoa number was numerically lower ($P>0.05$) for B, C and D diets versus the control diet. Results indicate that *Biophytum petersianum* Klotzsch can be used to modify rumen fermentation in order to decrease both ruminal ammonia concentration and protozoa number, thereby increasing microbial protein flow to the intestine.

Keywords: *Biophytum petersianum, saponin, ruminal fermentation, goats*

INTRODUCTION

Feeding silage-based diets to ruminants results in a peak in rumen ammonia concentration following meals (Thomas & Thomas, 1985). Excess ammonia in the rumen is absorbed into the blood stream, converted to urea in the liver and subsequently excreted in urine, thereby creating a negative environmental impact (Tamminga, 1992).

Increasing attention has been placed on use of natural products, instead of chemical feed additives such as antibiotics and ionophores, as manipulators of rumen fermentation. As stated by Russell & Rychlik (2001), there has been an increased perception that antibiotics and chemical compounds should not be routinely used as feed additives. The tropical plants containing saponins have been found to reduce ammonia production (Wallace et al., 1994; Takahashi et al., 2000; Santoso et al. 2004a). Accordingly, a slow release form of N to maintain a lower ammonia concentration might enhance fermentation by maintaining adequate rumen N for microbial growth after feeding. Saponins also have strong antiprotozoal activity and may serve as an

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effective defaunating agent (Wallace et al., 1994), thereby increasing microbial protein flow to the intestine and enhancing overall animal performance.

Biophytum petersianum Klotzsch (known locally as rumput Kebar) is an herb plant belonging to the Oxalidaceae family, which is distributed in Kebar District, Indonesia. It is not be planted by people but occurs and spreads naturally. The *Biophytum* plant has 10 – 16 pairs of leaf and may reach in height of 5 – 20 cm. Plant contains various secondary compounds such as steroidal saponin, flavonoid tannin and alkaloid.

The purpose of this study was to determine effect of *Biophytum petersianum* Klotzsch saponin on ruminal fermentation characteristics (i.e., pH, concentrations of ammonia-N and volatile fatty acids (VFA)), and protozoa number in goats fed a silage-based diet.

MATERIALS AND METHODS

Source of Biophytum and preparation of extract

Biophytum petersianum Klotzsch was collected from Kebar District, Indonesia. The area is located at an altitude of 500 - 600 m above sea level, 132°35' - 134°45' East longitude and 0°15' - 3°25' South latitude. All the parts of the plant (roots, stems and leaves) were used in this experiment. The plant was collected, dried under sunray until constant weight and milled through a 1.5 mm screen.

Biophytum aqueous extract was prepared based on method described by (Makkar et al., 1998). One liter of distilled water was added to 150 g of *Biophytum*, stirrer for about 3 h at room temperature using a magnetic stirrer, and filtered through two layers of cheesecloth. The filtrates were collected and stored at 4 °C for further use.

Animals, diets and experimental design

Four Kacang goats (indigenous breed of goat found in Indonesia) with an initial body weight (BW) of 20.25 ± 2.78 kg were kept in four individual metabolism cages and allocated in a 4 × 4 Latin square design. The animals were fed twice a day (08:00 and 16:00 h) at maintenance level (66 g DM/kg $BW^{0.75}$/day) with basal diet consisting of elephant grass silage and concentrate (70:30 on a DM basis; Table 1). The four treatments diets were basal diet (A), basal diet + 13 mg of saponin/kg BW (B), basal diet + 19 mg of saponin/kg BW (C), basal diet + 26 mg of saponin/kg BW (D). *Biophytum* aqueous extract was administered in amounts of 0, 160, 239 and 319 ml daily, which provided saponin at approximately 0, 13, 19.5 and 26 mg saponin/kg BW. The extract was given orally using a syringe twice daily at 08:00 and 16:00 (just after feeding). Silage was prepared from early bloom of elephant grass (*Pennisetum purpureum*). Commercial concentrate of CP 11 (Charoen Pokphand, Indonesia) was used in this experiment. Fresh water and a salt lick were available ad libitum. Before the start of the experiment goats were dewormed with 10 mg/kg BW of Kalbazen. Each experiment period lasted 14 days, and was comprised of 13 days for dietary adaptation followed by one day for rumen liquor sampling. Body weight of goats was weighed at the beginning and the end of each period.

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Rumen fluid collection

About 20 ml of rumen fluid was taken through the oesophagus by a flexible stomach tube and a vacuum pump at 0 h (just before feeding), and 1, 2, and 4 h after the morning feeding, subsequently squeezed through two layers of cheesecloth. The pH value was recorded immediately after sampling using a digital pH meter (HANNA, Hi 9321, Italy). For analysis of ammonia, 5 ml sub samples of filtrate were added to 5 ml of 20 ml/l (v/v) NaCl. Samples for determination of VFA were stabilized with 46 mM $HgCl_2$ solution (200 µl/1.8 ml rumen fluid). The mixtures were stored at –15 °C until analysis. Filtrate was also preserved for identification of protozoa by combining 1 ml filtrate with 0.1 ml of Hayem solution ($HgCl_2$, 2.5 mg/ml; Na_2SO_4, 25.0 mg/ml; NaCl, 5.0 mg/ml) (Hess et al., 2003). Protozoa was counted with the aid of a 0.1 mm depth Neubauer counting chamber (Clay-Adam, Parsippany, NJ).

Laboratory analyses

Samples of the feed offered and feces from each animal in each period were dried at 60 °C for 72 h in a forced-air oven, ground through a 1 mm mesh sieve and later analyzed for DM, ash and Kjeldahl N according to procedures of AOAC (1990). The neutral detergent fibre (NDF) was expressed on an ash-free basis without α-amylase and sodium sulphite (Van Soest et al., 1991). Acid detergent fibre (ADF) was analysed without adjustment for residual ash (AOAC, 1990). For the concentrate, α-amylase was used prior NDF analysis. The content of saponin was determined by TLC and in situ densitometry using a scanner. After separation on Silica gel GF_{254} using chloroform-ethanol (49:1 v/v) as mobile phase, the chromatographic zones corresponding to the spots were scanned at 301 nm. The concentration of ammonia N was essayed by the micro-diffusion modified by Conway and O'Malley (1942). Individual VFA were determined by capillary column gas chromatograph (Varian CP-9002 GC, Sunnyvale, CA).

Statistical analyses

The data were subjective to the analysis of variance for Latin Square design using the general linear model (GLM) procedure of SAS (SAS Inst., Inc., Cary, NC). Data on ruminal ammonia-N, pH and VFA collected at each sampling time were analyzed with the MIXED procedure of SAS for repeated measures. Significant differences between treatment means were determined by Duncan's multiple range test.

RESULT AND DISCUSSION

Chemical Composition of diet

The *Biophytum petersianum* Klotzsch plant contained 11 mg/g saponin. The saponin content was comparable to that of *Enterolobium cyclocarpum* (19 mg/g) or *Pithecellobium saman* (17 mg/g) but relative lower compared to *Yucca schidigera* (44 mg/g), *Sapindus saponaria* (200 mg/g) and alfalfa root (278 mg/g) as reported previously (Klita et al., 1996; Hristov et al., 1999; Hess et al., 2003).

Ruminal fermentation characteristics

Ruminal fermentation characteristics and protozoal population are in Table 2. Ruminal pH in goats given B, C, D diets was higher ($P<0.01$) compared to goats given

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A diet (control). Increased ruminal pH associated with saponin supplementation has been reported previously (Wilson et al., 1998; Santoso et al., 2004b).

Table 1. Chemical composition of elephant grass silage, concentrate and Biophytum petersianum Klotzsch

	Silage	Concentrate[a]	Mixed diet[b]	*Biophytum* sp.
Dry matter (g/kg)	264	882	450	441
Organic matter (g/kg DM)	929	937	931	868
Crude protein (g/kg DM)	60	222	109	80
NDF (g/kg DM)	760	162	581	589
ADF (g/kg DM)	580	93	444	506
Hemiselulose (g/kg DM)	180	69	145	83
Crude saponin (g/kg DM)	-	-	-	11

[a] Contained corn, rice bran, fish meal, soybean meal, coconut meal, bone meal, wheat, peanut meal, canola, leaf meal, vitamins, calcium, phosphate and trace minerals.
[b] 70% elephant grass silage and 30% concentrate on a DM basis

The higher pH values in goat given saponin may reflect a decrease in total VFA concentration that presumably could be due to lower activity of holotrich protozoa. Coleman (1979) indicated that holotrich protozoa are able to rapidly ferment soluble sugars and produce VFA and lactic acid. However, our pH values were above 6.0 required for microbial protein synthesis (Russell et al., 1992).

Ammonia concentration in the rumen is a balance between degradation of feed protein and uptake of ammonia for the synthesis microbial protein. The ruminal ammonia-N concentration peaked 1 h after feeding in all treatments (Figure 1), and saponin-supplemented diets (B, C, D) had consistently lower concentrations ($P<0.01$) at all times. A number studies with sheep fed silage-based diet (Santoso et al., 2004a, 2004b) and *in vitro* ruminal culture fermentations (Wallace et al., 1994) noted that saponin of *Y. schidigera* effectively reduce the concentration ammonia concentration. It has been reported (Williams & Coleman, 1991) that reduced ammonia concentrations in the rumen are typical when protozoal growth is inhibited, presumably as a result of depressed rumen degradation of feed protein. Another possible explanation for the reduction in ruminal ammonia in saponin-supplemented goats may have been due to increased incorporation of ammonia, peptide or amino acids into microbial protein.

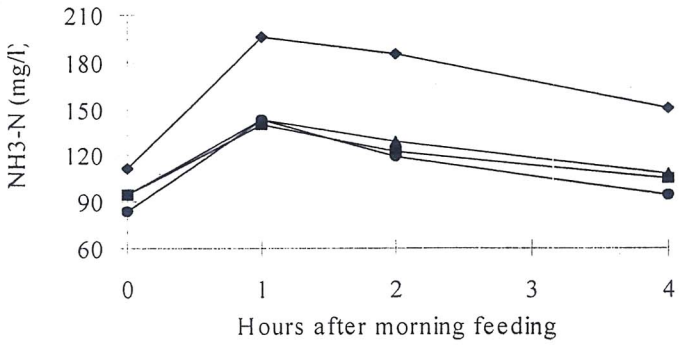

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Figure 1. *Ruminal ammonia N concentration in goats fed a silage-based diet supplemented with saponin of Biophytum sp. at 0 (♦), 13 (▲), 19.5 (■) and 26 (●) mg/kg BW.*

Table 2. Effects of saponin from Biophytum petersianum Klotzsch on ruminal fermentation characteristics and protozoal populations in goats.

	Saponin dose (mg/kg BW)				S.E.M	P-value
	0	13	19.5	26		
pH	6.91[b]	7.01[a]	7.02[a]	7.02[a]	0.021	<0.01
Ammonia N (mg/l)	161.0[a]	119.0[b]	115.5[b]	110.2[b]	5.87	<0.01
Total VFA (mM)	79.3	72.2	68.2	63.1	4.35	0.14
Individual VFAs (mol/100 mol)						
Acetate	68.1[a]	61.5[c]	62.1[bc]	65.7[ab]	0.95	<0.01
Propionate	21.9[c]	28.7[a]	29.2[a]	25.5[b]	0.81	<0.01
Butyrate	6.4	6.3	5.6	5.7	0.27	0.12
Valerate	0.6	0.6	0.5	0.5	0.07	0.48
iso-Acids[a]	3.0	2.8	2.6	2.5	0.15	0.15
Protozoa (10^4/ml)	13.6	8.9	8.2	8.0	1.71	0.17

Means within a row with different superscripts differ ($P<0.01$).
[a]Includes *iso*-butyrate and *iso*-valerate

Total VFA concentration, proportions of butyrate, valerate and *iso*-acids were similar (P>0.05) among saponin treatments. The numerically lower total VFA concentration in response to increasing saponin concentration might be due to decreased microbial VFA production particularly by protozoa. According to Coleman (1979) holotrich protozoa are able to rapidly ferment soluble sugars and produce VFA and lactic acid. No significant difference in total VFA concentration with supplementation of saponin (Klita et al., 1996; Hristov et al., 1999; Santoso et al., 2004a) has been reported. This is in contrast with results of the study by Lu & Jorgennsen (1987), in which a reduction in VFA concentrations was observed when saponin was administrated at 4% of dietary intake.

Goats fed the control diet had higher (P<0.01) proportion of acetate compared to goats fed B and C diets. Proportion of propionate was significantly different (P<0.01) among treatments. It was the highest in goats receiving D diet and the lowest in goats receiving A diet. Increase in molar proportion of propionate in the rumen by extract *Biophytum* could be due to presence of saponin and its inhibitory effect of protozoa, which is in agreement with previous studies (Kil et al., 1994; Hristov et al., 1999; Santoso et al., 2004b). Moreover, the growth of propionate-producing bacteria i.e. *Selemonas ruminantium* was not affected by yucca saponin, whereas growth of some other rumen bacteria species i.e. *Streptococcus bovis* and *Butyrivibrio fibrisolvens* was strongly inhibited (Wallace et al., 1994). In the present study, however, there was no obvious trend in molar proportions of acetate and propionate pattern with increasing saponin from *Biophytum* sp.

Protozoal populations in the rumen was numerically lower (P=0.17), however, with saponin treatments than with control (by 34.6, 39.7 and 41.2%, respectively with B, C and D diets), but due to large variation, the difference was not statistically significant. Reduced protozoal population with increasing doses of saponin agrees with

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previous reports (Lu & Jorgensen, 1987; Wallace et al., 1994; Klita et al., 1996). The detergent action of saponin kills rumen protozoa. The susceptibility of rumen protozoa and lack of susceptibility of rumen bacteria to saponin could be due to presence of cholesterol in eukaryotic membranes (including protozoa) but not in prokaryotic bacterial cells (Klita et al., 1996).

CONCLUSION

The saponin of *Biophytum petersianum* Klotzsch has antimicrobial properties, particularly in suppressing protozoa number, which lead indirectly to lower ruminal ammonia concentration. Reducing protozoa number could improve the microbial yield in the rumen and protein flow to the animal. The results of this experiment indicate that the *Biophytum petersianum* Klotzsch extract appear to have a potential to manipulate rumen fermentation in order to improve ruminant performance.

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