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EFFECT OF LERAK (*SAPINDUS RARAK*) EXTRACT IN HIGH ROUGHAGE DIET ON RUMEN MICROBIAL PROTEIN SYNTHESIS AND PERFORMANCE OF SHEEP

*Pengaruh Ekstrak Lerak (*Sapindus Rarak*) Dalam Pakan Serat Tinggi Terhadap Sintesis Protein Mikroba Rumen Dan Performa Domba*

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ABSTRACT

Secondary compounds containing plant extract as feed additive may improve the performance of livestock consuming high roughage diet. An *in vivo* trial was conducted to investigate the effect of *Sapindus rarak* extract (SRE) on ruminal fermentation products, microbial protein synthesis, and growth performance of sheep. Sheep (male, 28 heads) fed high roughage diet were arranged in a completely randomized design with four treatments: addition of SRE to the diet at 0, 4, 8 and 12 g head^{-1} day^{-1}. The experiment was conducted for 105 days with 2 weeks adaptation period. At the end of the experiment, total faeces was collected for 1 week and rumen liquor was taken. Variables measured were ruminal fermentation products, microbial protein synthesis, daily intake, digestibility, N retention, body weight, and average daily gain. Protozoal numbers were significantly decreased with increasing SRE dose. The ruminal NH_3 concentration tended to reduce by SRE addition ($P = 0.06$). SRE significantly increased propionate and efficiency of microbial protein synthesis from 6.4 to 10.5 g N kg^{-1} DOMR. SRE significantly improved average daily gain of sheep during the first 70 days of experiment without affecting intake and digestibility. SRE did not alter carcass percentage but tended to lower pancreas and liver weights ($P<0.1$). In conclusion, SRE has a significant role in partially defaunated rumen microflora, hence, increased microbial protein synthesis and propionate production in the rumen. Addition of SRE is useful to increase daily gain of sheep fed high roughage diet in 70 days of feeding.

[**Keywords**: growth, microbial protein synthesis, saponin, *Sapindus rarak*, sheep]

ABSTRAK

Ekstrak tanaman yang mengandung senyawa sekunder dapat meningkatkan performa ternak yang mengonsumsi pakan berserat tinggi. Percobaan in vivo dilakukan untuk mengevaluasi pengaruh ekstrak saponin lerak (Sapindus rarak) (*SRE*) *pada produk fermentasi rumen, sintesis protein mikroba, dan kinerja pertumbuhan domba. Domba (jantan, 28 ekor) yang diberi pakan serat kasar tinggi ditempatkan dalam rancangan acak lengkap dengan empat perlakuan pemberian SRE ke dalam ransum dengan dosis 0, 4, 8, dan 12 g ekor^{-1} hari^{-1}, percobaan dilakukan selama 105 hari dengan periode adaptasi 2 minggu. Pada akhir percobaan, feses dan urine domba dikumpulkan selama 1 minggu dan cairan rumen diambil. Variabel yang diukur yaitu produk rumen fermentasi dan sintesis protein mikroba, konsumsi harian, kecernaan, retensi N, bobot badan, dan pertambahan bobot badan harian. Jumlah protozoa berkurang secara signifikan oleh SRE selaras dengan meningkatnya dosis. Konsentrasi amonia dalam rumen cenderung menurun dengan penambahan SRE* ($P = 0,06$)*. Ekstrak saponin lerak meningkatkan propionat secara signifikan dan. efisiensi sintesis protein mikroba dari 6,4 menjadi 10,5 g N kg^{-1} DOMR. SRE secara signifikan meningkatkan pertambahan bobot badan harian domba selama 70 hari pertama percobaan tanpa memengaruhi konsumsi dan kecernaan pakan. SRE tidak mengubah persentase karkas, tetapi cenderung menurunkan bobot pankreas dan hati* ($P <0,1$)*. Dengan demikian, SRE memiliki peran penting dalam men-defaunasi sebagian mikroflora rumen, sehingga meningkatkan sintesis protein mikroba dan produksi asam propionat di dalam rumen. Penambahan SRE bermanfaat untuk meningkatkan pertambahan bobot badan domba yang diberi pakan serat tinggi dalam pemeliharaan selama 70 hari.*

[**Kata kunci**: pertumbuhan, sintesis protein mikroba, saponin, *Sapindus rarak*, domba]

INTRODUCTION

In tropical countries, most feed especially during dry season contains limited amount of nitrogen that prevent rumen microorganism to have optimum growth. Feeding high roughage diet to their livestock has been practised by small farmers, hence, livestock performance usually in poor conditions especially during long dry season.

Feed additive is one of many strategies that could improve livestock performance. Plant extracts which contain secondary compounds have been reported to enhance livestock performance. Saponin which is one of the secondary compounds has the ability to defaunate

the rumen by binding the sterol in protozoal membranes and lysed the protozoa. Protozoa in the rumen predates on bacteria causing further losses of microbial protein in the rumen, hence, less synthesis of microbial protein in the rumen and less nitrogen flow to duodenum. Several reports on the effects of saponins on rumen fermentation and feed digestibility are available (Wina 2012); Jayanegara et al. 2014; Liu et al. 2019).

One of saponin sources available in Indonesia is found in the fruit of *Sapindus rarak* known as soapnut or *lerak*. *Lerak* has been used widely in Indonesia as traditional soap for batik clothes. Saponin is the major compound in *lerak* fruit that caused foaming properties when the fruits were mixed in the water. As the bioactive compound, saponin content in *lerak* fruit was very high (32.54-35.98%) as reported by Pasaribu et al. (2014).

Experiments have been conducted to observe the effect of *Sapindus rarak* extract (SRE) on *in vitro* fermentation, the effect of interval feeding of SRE on feed digestibility (Wina et al. 2006b), and the effect of short and long term feeding of SRE on fibrolytic rumen microorganism (Wina et al. 2006a). However, there were only several data available on the effect of Sapindus saponins on microbial protein synthesis in the rumen, organ weight, and growth performance of livestock fed high roughage diet (Wang et al. 2012; Liu et al. 2019).

The objective of this study was to investigate the effect of saponins-containing methanol extract of SRE on ruminal fermentation products, microbial protein synthesis, organ weight, and growth performance of sheep consuming high roughage diet.

MATERIALS AND METHODS

Preparation of Methanol Extract from Sapindus rarak

Sapindus rarak fruits (from Central Java, Indonesia) were dried in the oven at 60°C, the the dried fruit powder was soaked with methanol (1:4w/v) for 16 hours. The extracts were separated from the residue, then the residue was soaked again with methanol for another 16 hours. The combined extracts were evaporated by rotary evaporator (Wina et al. 2006a). Then, the concentrated liquid was freeze dried. The products were very hygroscopic and had to be kept in airtight bag until used.

Feeding Experiment

Twenty-eight male sheep (thin tailed local Indonesian breed) with an average body weight of 16.8±1.9 kg, about 1-1.5 year old were kept in individual wooden slatted cage, about 0.75 m above the ground. The sheep were adapted to a mixture diet containing sugarcane tops and wheat pollard in the ratio of 65:35 w/w for 2 weeks. The diet was arranged as low quality diet and did not follow the nutrient requirement of the sheep.

The experiment had four treatments of feeding different levels of SRE, i.e. 0 (control without addition of SRE), 4, 8 and 12 g SRE head^{-1} day^{-1}. SRE was added to wheat pollard and was offered before the sugarcane tops to ensure complete uptake of the saponin extract. The proximate composition of sugarcane tops and wheat pollard is presented in Table 1. Crude protein, neutral detergent fibre, and gross energy content of the diet were 92.3 g kg^{-1}, 646 g kg^{-1} and 15.3 MJ kg^{-1} DM respectively. Calcium in the form of limestone and salt were added at the level of 12 g kg^{-1} and 3.3 g kg^{-1}, respectively The diets were offered to the animals for 105 days at the level of 4% of body weight. Animals had free access to drinking water except during the digestibility trials where 2 liters of water in a bucket were offered daily. The body weight of sheep was recorded every second week before morning feeding.

Sample Collection

Faeces and urine collections were done according to the method described in IAEA protocol (International Atomic Energy Agency 1997). Faeces were collected every day for two periods of 7 days each; those periods were from day 28 to day 35 and from day 70 to day 77 of the feeding experiment. A bulk of faeces was weighed every morning before morning meal and a representative subsample (about 10%) was taken, oven dried at 60°C and the weight was recorded. Daily samples were then combined for further analysis.

Urine was collected every morning in the same period of faeces collection. It was collected in a bucket containing 100 ml of 10% sulphuric acid solution. Volume was made up to 2 liters with water and an aliquot was taken and stored at –20°C. Daily samples from each animal were then pooled at the end of the trial

Table 1. Proximate composition of the dietary components.

	Sugar cane tops	Wheat pollard
Organic matter (% DM)	91.1	95.2
Nitrogen (% DM)	0.92	2.51
Fat (% DM)	0.70	0.57
NDF (% DM)	82.1	32.2
ADF (% DM)	46.9	9.57
Lignin (% DM)	6.34	2.15
Gross energy (MJ kg^{-1})	1.46	1.65
Calcium (% DM)	0.42	0.10
Phosphor (% DM)	0.10	0.68

and were analysed for purine derivative and nitrogen concentrations.

Rumen liquor (50 ml) was taken from each sheep using a plastic stomach tube and filtered through two layers of cheese cloth. Rumen liquor was taken twice at day 103 and day 105 (the last day of the experiment).

For short chain fatty acid (SCFA) analysis, an aliquot of 1.5 ml was centrifuged at 14,000 g for 10 minutes. The supernatant (0.9 ml) was mixed with 0.1 ml of formic acid containing internal standard (10 ml l^{-1} of methyl valeric acid) and kept at 4° C overnight. The next day, the mixture was centrifuged at 14,000 g for 10 minutes and the clear supernatant was transferred into a glass vial and kept at 4°C for further analysis.

An aliquot (0.3 ml) of the filtered rumen liquor was collected into a vial containing the fixative reagent for protozoal counts and stored at 4°C. Another aliquot (1 ml) of the filtered rumen liquor was collected for ammonia determination, which was done immediately using the Conway method (Conway and Byrne 1933)

Chemical Analysis

Feed, feed residues and faeces were subjected to analysis of DM (24 h at 105°C) and total ash (Association of Official Analytical Chemists 1984), nitrogen, NDF, and ADF lignin. Nitrogen content was determined by Kjeldahl and nitrogen released after digestion was then analysed by autoanalyser. The NDF content of sugar cane tops and faeces were determined without amylase and a NDF content of wheat pollard was analysed with heat stable α-amylase using Van Soest et al. (1991) method. The NDF values were not corrected for ash content. Gross energy was measured using bomb calorimeter.

SCFA were analysed by gas chromatography (GC-14A, Shimadzu Corp, Japan Tokyo) fitted with a flame ionization detector. Separation was carried out in a stainless steel column packed with GP 107, SP 1000/1 % H_3PO_4 on Chromosorb WAW (100/120 mesh) (Hoeltershinken et al. 1997).

Protozoa were fixed in a solution containing 4% formaldehyde 13.5 mM NaCl and 0.6 mg ml^{-1} methyl green and counted in a Neubauer Chamber. The data of protozoal count were presented in $\log_{10}$. Log reduction (L) = $\log_{10}$ (control) - $\log_{10}$ (treatment).

Percent protozoa reduction (P)= $(1\text{-}10^{-L})*100$

Concentrations of allantoin, uric acid, xanthine and hypoxanthine in the urine were determined by the method described in (Chen and Gomes 1992).

Calculation of Microbial Protein Synthesis

Microbial protein synthesis and efficiency of microbial protein synthesis were calculated by formula as described in (Chen and Gomes 1992) based on the total purine derivatives excretion in the urine.

Excretion of purine derivatives (Y): Y (mmol d^{-1}) = allantoin + uric acid + (xanthine + hypoxanthine)

Absorption of microbial purines (**X**): Y (mmol d^{-1}) = 0.84 **X** + (0.150 $W^{0.75}$ $e^{-0.25X}$)

$$\text{Microbial N synthesis (g N day}^{-1}) = \frac{X \text{ (mmol d}^{-1}) \times 70}{0.83 \times 0.116 \times 1000} = 0.727\ X$$

Notes: The digestibility of microbial purines is assumed to be 0.83.

The N content of purines is 70 mg N $mmol^{-1}$.

The ratio of purine N : total N in mixed rumen microbes is taken as 11.6 : 100.

Efficiency of microbial protein synthesis (g N kg^{-1} DOMR) = microbial N synthesis $DOMR^{-1}$

Where DOMR (digestible organic matter apparently fermented in the rumen) = 0.65 of the DOMI (digestible organic matter intake)

Weight Measurement of Carcass, Skin, Organs, and Fat Deposit Around the Organs

All sheep were slaughtered at the end of experiment (day 105). The sheep were slaughtered 3 hours after morning feeding. The carcass and skin weights were recorded. The fat covering the organs was collected and weighed separately. Empty body mass (EBM) was calculated by subtracting the weight of gut contents from the body weight. The carcass, organs and organ fat weights were expressed as g kg^{-1} EBM.

Statistical Analysis

Values of digestibility are the average from two periods of faeces and urine collection. The values were then analysed using GLM procedure in SAS package version 8. Means were compared by Duncan's multiple range test.

RESULTS

Rumen Parameters

Total short chain fatty acid (SCFA) concentration in the rumen was not affected by SRE (Table 2). Total SCFA concentration was the sum of all individual SCFA. The

Tabel 2. Ruminal fermentation products of sheep fed different levels of *Sapindus rarak* extract (SRE).

Parameter	SRE level (g day^{-1})				sem	P value
	0	4	8	12		
SCFA (µmol ml^{-1})	68.77	59.22	66.75	64.41	4.04	0.1468
Acetate (mol %)	73.59^{a}	71.06^{b}	73.94^{a}	73.46^{a}	0.71	0.0184
Propionate (mol %)	14.68^{a}	16.66^{b}	15.93^{b}	17.47^{c}	0.44	0.0003
Butyrate (mol %)	9.05^{b}	9.18^{b}	7.68^{a}	6.45^{a}	0.46	0.0001
Isobutyrate (mol %)	0.9	1.05	0.88	0.9	0.07	0.2312
Valerate (mol %)	0.60^{c}	0.57^{cb}	0.46^{a}	0.49^{ab}	0.03	0.0113
Isovalerate (mol %)	1.17	1.48	1.12	1.23	0.12	0.1513
Ammonia (mg ml^{-1})	0.33	0.29	0.28	0.27	0.02	0.0646

Different letter in the same row denotes significant difference (P<0.05).
sem = standard error for means.

high molar proportion of acetate compared to other SCFA indicated the normal pattern of high forage diet. However, the proportions of the individual SCFA changed significantly with inclusion of different levels of SRE. A significant increase (P<0.05) of propionate was obtained with SRE inclusion. However, a significant decrease (P<0.05) of acetate, butyrate and valerate productions and no significant change in the proportion of branched chain fatty acid (isobuyrate and isovalerate) were found in the presence of SRE (P>0.05).

Addition of SRE linearly decreased ruminal ammonia concentration. The concentration at the highest inclusion of SRE tended to reduce ammonia by 18% from that of control treatment (P = 0.06, Table 2). SRE significantly decreased protozoal counts in a dose dependent manner with high correlation between protozoal count to dose of SRE addition (r^2 = 0.9865). Compared to control, the protozoal count reduction were 33.9%, 60.2% and 80.0% at the addition of 4, 8 and 12 g SRE head^{-1} day^{-1}, respectively.

Intake, Digestibility, and Microbial N Synthesis

Addition of SRE increased DM roughage intake about 11–13.9% compared to control treatment, but the total DM intake and other nutrient intake in gram per day were not significantly different (P>0.05) among treatments (Table 3). Compared to control, the highest inclusion level of SRE (12 g head^{-1} day^{-1}) slightly reduced the digestibilities of organic matter, neutral detergent fibre and nitrogen of the diet by 4.5%, 4.3% and 8.0%, respectively, however, these differences were not significant (P>0.05) among treatments.

Based on purine derivatives excretion in urine, the microbial N synthesis and efficiency of microbial N synthesis were calculated. The result in Figure 1 shows an increase of both parameters in a dose dependent manner with high correlation (r^2 = 0.9038 and 0.9376, respectively).

Average Daily Gain and Feed Conversion Ratio (FCR)

Figure 2 shows the average body weight of sheep fed different levels of SRE for 105 days. From the first week of experiment, there was a difference in average body weight between treatments of 8 and12 g SRE head^{-1} day^{-1} and treatments of 0 and 4 g SRE head^{-1} day^{-1}. The difference became bigger up 70 days of experiment. The curve starts to plateau after 70 days of feeding experiment.

Table 4 shows that the lowest level of SRE had no influence on growth performance. However, the addition of higher levels of SRE (8 and 12 g head^{-1} day^{-1}) significantly increased average daily gain by 37% and 44%, respectively up to 70 days, but not up to 105 days of experiment. Feed conversion ratio (FCR) at 0–70 days decreased by 30% and 28% for sheep fed 8 and 12 g SRE head^{-1} day^{-1} of SRE, respectively, but was not significantly different from that of control. The FCR at 0–105 days was similar among treatments.

The Weights of Carcass, Skin, Fat Deposits Around Organs and Organs

No significant difference was found in the weights of carcass and skin for all treatments (Table 5). The total fat deposits around the organs were also not significantly different among treatments. SRE did not cause any significant difference in all organs weights. However, pancreas and liver weights tended to reduce in a dose dependent manner (P <0.1).

DISCUSSION

Effect of SRE on Rumen Fermentation

SRE exerted a persistent effect on suppressing protozoa numbers in a dose dependent manner up to 105 days with high correlation (r^2 = 0.9865) between level of SRE and

Table 3. Feed intakes and digestibilities on sheep fed different levels of *Sapindus rarak* extract (SRE).

Parameter	SRE level (g day^{-1})				sem	P value
	0	4	8	12		
Intake (g day^{-1})						
DM roughage	310	344	353	348	29.3	0.7240
DM concentrate	229	232	235	238	9.7	0.9068
DM total	539	575	588	586	35.7	0.7417
OM total	501	535	547	546	33.1	0.7401
NDF total	323	352	361	358	26.4	0.7223
N total	9	9.34	9.48	9.52	0.4	0.8233
Digestibility (%)						
DM	59.9	58.6	56.7	56.8	15.5	0.4390
OM	62.3	61.1	59.3	59.5	14.8	0.4339
NDF	49.3	49.5	47.2	47.2	21.6	0.7987
N	65.3	63.0	60.5	60.1	16.1	0.1052

Note: sem = standard error for means.

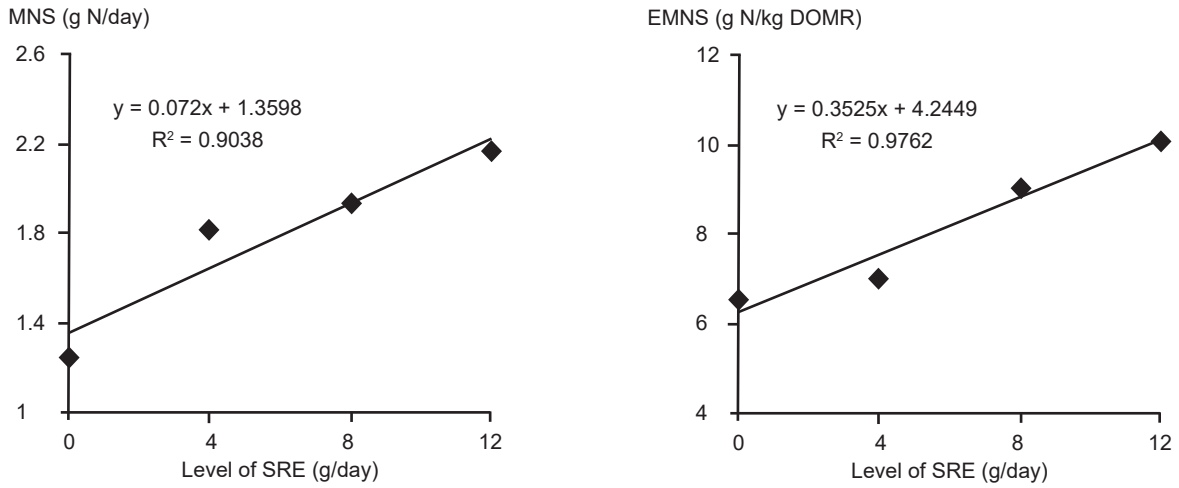

Fig. 1. Microbial N synthesis (MNS) and efficiency of microbial N synthesis (EMNS) in sheep fed different levels of *Sapindus rarak* saponin extract (SRE). DOMR (digestible organic matter apparently fermented in the rumen) = 0.65 of the DOMI (digestible organic matter intake).

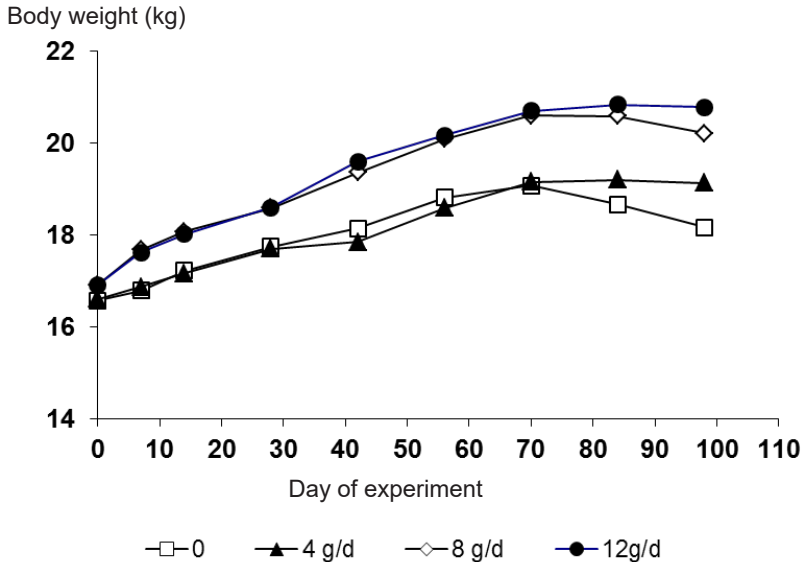

Fig. 2. Average body weight of sheep fed high roughage diet with different levels of *Sapindus rarak* saponin extract for 105 days.

Table 4. Body weight, daily weight gain, and feed conversion ratio (FCR) of sheep fed different levels of *Sapindus rarak* extract (SRE).

Parameter	SRE level (g day^{-1})				Sem	P value
	0	4	8	12		
Initial body weight (kg)	16.6	16.6	16.9	16.9	0.8	0.9879
Final body weight at 105 days (kg)	18.8	19.6	20.4	20.8	1.0	0.4999
Average daily weight gain (g day^{-1})						
0-70 days	36.9^{b}	35^{b}	50.5^{ab}	53.2^{a}	5.3	0.0500
0-105 days	25.0	27.8	32.7	37.3	7.2	0.6108
FCR						
0-70 days	17.0	17.8	11.9	12.3	2.1	0.1416
0-105 days	21.5	33.3	29.9	21.4	8.6	0.6754

Different letter in the same row denotes significant difference (P<0.05).
sem = standard error for means.

Table 5. Carcass, organs and total fat deposit around the organs of sheep fed different levels of *Sapindus rarak* extract (SRE).

Parameter	SRE level (g day^{-1})				Sem	P value
	0	4	8	12		
Carcass (g kg^{-1} EBM)	541	547	522	541	11.08	0.4252
Skin (g kg^{-1} EBM)	88.2	104	92.7	104	6.69	0.2615
Organ fat (g kg^{-1} EBM)	26	28	30	27	2.87	0.8415
Heart (g kg^{-1} EBM)	4.7	4.5	5.5	4.6	0.42	0.3729
Kidney (g kg^{-1} EBM)	3.6	3.3	3.1	3.1	0.2	0.3536
Liver‡ (g kg^{-1} EBM)	17.1	16.8	16.3	14.4	0.74	0.0602
Spleen (g kg^{-1} EBM)	2.8	2.9	2.5	2.7	0.22	0.6674
Pancreas (g kg^{-1} EBM)‡	2.2	1.9	1.5	1.2	0.28	0.0654

EBM = empty body mass .
Organ fat is the sum of the fat around heart, kidney, testis, mesenteric tissue and omentum.
‡ approaching significant (P<0.1).
sem = standard error for means.

protozoal count in the rumen. The ability to reduce protozoal count is due to the ability of saponin in SRE to interact with cholesterol in the outer membrane of the protozoa, causing lysis of the protozoal membrane (Wina 2012). SRE seemed to be one of the saponin sources that partially defaunate the rumen consistently both on the *in vitro* experiment (Wina et al. 2006b; Suharti et al. 2011) and also sheep for short term and over 3 months (Wina et al. 2006b). In the *in vivo* trials, antiprotozoal effect of other saponins tend to disappear after 7–14 days (Patra and Saxena 2009).

Other saponin such as tea seed saponin has various effects. It had antiprotozoal effect on the *in vitro* rumen fermentation on sheep (Wang et al. 2012), but not on dairy cow (Guyader et al. 2017). The long term effect to reduce protozoal count may be caused by the presence of 20 different structures of saponins that have been isolated and identified in *S. rarak* pericarp's extract (Asao et al. 2009). These saponins have the same sapogenin and hederagenin, with different amounts and types of sugars attached to it and they may have different antiprotozoal activities and time of degradation in the rumen. (Ramos-Morales et al. 2019) reported that the same sapogenin with different amounts and types of sugar such as saikosaponin A, B, and C had different activities against protozoa in the rumen. Previously, (Ramos-Morales et al. 2017) suggested that a persistent effect to depress protozoa can be done by chemical modification of the group that attached to the sapogenin. The type of diet fed to the animal, besides the saponin structure may also affect the antiprotozoal activity of these saponins (Patra and Saxena 2009; Dai and Faciola 2019). In this experiment, types of protozoa that survived in the presence of SRE were not identified, but (Zhou et al. 2011) showed that tea saponin could selectively inhibit specific type of protozoa causing reduction of protozoal diversity in the rumen of sheep.

In this experiment, the major effects of SRE on ruminal fermentation were a shift of SCFA pattern towards an increased proportion of propionate and a decreased butyrate and valerate. The higher propionate proportion may be the result of lower acetate and butyrate, which are the major fermentation end products of protozoa. Therefore, when the number of prozotoa was depressed by saponin, a significant increase in the proportion of propionate would be expected. The increased propionate may also due to the increased population of *Prevotella ruminicola* which produce propionate and succinate in the rumen as reported by (Suharti et al. 2011) in their study on the effect of SRE in the *in vitro* fermentation using cattle rumen fluid. With higher propionate production in the rumen, there will be less hidrogen available for methane production, hence, less methane produced by sheep fed

SRE (Thalib et al. 2010 ; Yulistiani et al. 2017). With higher propionate and lower acetate obtained in this experiment, it may lead to more energy available for nutrient utilization and animal growth as propionate is the most important precursor of gluconeogenesis (Martínez-Aispuro et al. 2018).

As a consequence of partly defaunation, ruminal ammonia concentrations tended to decrease by SRE addition (Table 2). With less protozoa in the rumen, lower turn over protozoa occurred, hence, less ammonia from protozoa lysis was produced in the rumen since protozoa contributed some 10–40% of the total rumen nitrogen. The decreased ammonia production may be also due to the lower bacterial protein breakdown by protozoa and lower feed protein degradability in the rumen (Newbold et al. 2015). Although ammonia concentration decreased, its concentration in the rumen was not critical since it was still close to the value needed for the optimum microbial growth (0.15–0.25 mg N ml^{-1}).

Microbial protein synthesis and efficiency of microbial protein synthesis that were estimated from purine derivative analysis increased linearly with an increasing level of SRE (Figure 1). The higher the level of SRE added into the feed, the higher the microbial protein synthesis and efficiency of microbial protein synthesis. The same result was reported with tea saponin when fed sheep with 3 g $head^{-1}$ day^{-1} of tea saponin (Mao et al. 2010) and when fed goat with 0.4% DM intake day^{-1} of tea saponin (Kumar et al. 2017). As saponins reduced protozoa, a lower predation of bacteria by protozoa occurred and resulted in a higher number of bacterial population or higher microbial protein synthesis and a slower microbial protein turnover in the rumen leading to increase bacterial N flow to the duodenum (Belanche et al. 2011). This bacterial protein that flow to the duodenum will be hydrolysed to amino acid for building block for protein and muscular development body during sheep growth (Lopes et al. 2019). The low quality of forage will only contribute low nitrogen for microbial protein synthesis in the rumen and the addition of SRE would be beneficial for low quality forage diet.

Effect of SRE on Animal Performance

Addition of SRE 12 g $head^{-1}$ day^{-1} to wheat pollard did not affect the palatability and consumption of concentrate or grasses. Saponin was reported to have bitter taste and bitter taste would reduce feed intake (Favreau et al. 2010). Favreau et al. (2010) used quinine solution as the bitter compound at the level of 2–3 g^{-1} kg sprayed on the hay, but at this experiment, the bitter taste of 4–12 g SE day^{-1} may be disappeared after mixing with wheat pollard and did not cause any reduction in daily intake of feed. There were also no reduction of DM, OM, NDF digestibilities or nitrogen retention by addition of 4–12 g (0.7–2 g SE 100^{-1} g feed consumption). The results were similar to those reported by Yulistiani et al. (2017) that the addition of 1–2% complete rumen modifier (CRM) which also contained *S. rarak* pericarp powder did not affect DM consumption, DM, OM, NDF digestibilities, but with higher inclusion (3% CRM), DM, OM and NDF digestibility was significantly reduced compared to control (without CRM). Protozoa have several fibrolytic enzymes (Newbold et al. 2015), therefore NDF and ADF digestibilities reduced with elimination of protozoa but those perhaps were not affected with partly defaunation.

SRE increased the body weight gain of male sheep (Table 4). It was reported that saponins enhanced the performance of ruminants fed roughage-based diets (Patra and Saxena 2009). However, various results were reported on the effect of saponin on growth performance. Complete rumen modifier that contained *S. rarak* fruit powder had a significantly positive effect on weight gain of sheep fed rice straw diet (Thalib et al. 2010), but not significant when sheep fed corn cob silage compared to control (Yulistiani et al. 2017). *S. rarak* fruit extract when fed to Ongole cattle at the level of 100-200 mg kg^{-1} feed caused only a trend increase of body weight gain (Suharti et al. 2015). Other saponin source, tea saponin, significantly increased weight gain of goat fed at the level of 0.4% DM intake for 120 days (Kumar et al. 2017). The effect of SRE on body weight gain of sheep was observed immediately and significantly at the early weeks up to 70 days of feeding trial, but not significant up to 105 days of feeding trial. The variations among animals in this experiment were quite high and the weight gain of the animals to 105 days of experiment became smaller and was not signficantly different among treatments.

It was reported by Patra and Saxena (2009) that the different types and levels of saponins and also the different other secondary compounds in the saponin extract or saponin containing plant materials may affect the various results of the effect of saponin on growth performance. It may also be due to the different compositions, levels of protein and energy of diet .

There are very limited data on the effect of saponins on carcass weight. In the present experiment, the carcass weight was not affected by SRE feeding. The percentage of carcass to the empty body weight was in average of 54%. Sen et al. (2000) reported that total defaunation did not cause any difference in carcass weight and the proportion of carcass to the empty body mass was 52%. Eugène et al. (2004) summarised the data from 17 trials and reported that defaunation increased the ruminal volume, which might contribute to the increase in weight gain of

defaunated animal. The fat deposits were not different for all treatments (Table 5) indicating that the increase of body mass gain was not distributed to the fat deposit around organ tissues.

Feed conversion ratio up to 70 days was reduced by 30% when sheep was fed 8 g SRE head^{-1} day^{-1} suggesting that saponin-fed sheep may be more efficient in the utilization of feed than control sheep. Newbold et al. (2015) reported that the efficiency of energy utilization for growth improved even though body energy retention did not increase.

In this experiment, the increasing level of SRE added to the diet seemed to cause smaller weights of pancreas and liver, but these weights were not significantly different among treatments. The occurrence of liver damage has been reported on the animal that suffered from photosensitization caused by some saponin containing plant materials (Low 2015). Saponin that ingested into digestive tract will be degraded in the rumen and the sapogenin may be modified or transformed to conjugated sapogenins that present in the bile and liver of animals and cause some cells damaged in liver and kidney (Low 2015). Sheep during feeding trial were inside the animal house, therefore there was no report on photosensitization case in this experiment. Smaller weight of pancreas and liver may not affect growth since higher weight gain was obtained with higher SRE level addition up to 70 days of trial.

Saponin was reported to inhibit lipase activity produced in pancreas (Marrelli et al. 2016), however, the relation between lipase activity inhibition and smaller size of pancreas has not yet been clear. Inhibition of lipase activity resulted in lower cholesterol in blood. In the present study, we did not find any visual changes in the outside appearance of the liver, kidney or pancreas. Further study on the effect of *S. rarak* saponin extract on the health of the animal is needed.

CONCLUSION

Sapindus rarak saponin extract has a persistent effect on suppressing the protozoal numbers and shift SCFA profile. *Sapindus rarak* saponin extract increased weight gain of the animals fed high roughage diet up to 70 days of experiment by enhancing microbial protein synthesis and molar proportion of propionate.

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REFERENCES

Asao, Y., Morikawa, T., Xie, Y., Okamoto, M., Hamao, M., Matsuda, H., Muraoka, O., Yuan, D. & Yoshikawa, M. (2009) Structures of acetylated oleanane-type triterpene saponins, rarasaponins IV, V, and VI, and anti-hyperlipidemic constituents from the pericarps of *Sapindus rarak*. *Chemical & Pharmuceutical Bulletin*. [Online] 57 (2), 198–203. Available from: doi:10.1248/cpb.57.198.

Association of Official Analytical Chemists (1984) *Official Methods of Analysis*. 14th edition Arlington, VA, AOAC.

Belanche, A., Abecia, L., Holtrop, G., Guada, J.A., Castrillo, C., de la Fuente, G. & Balcells, J. (2011) Study of the effect of presence or absence of protozoa on rumen fermentation and microbial protein contribution to the chyme1. *Journal of Animal Science*. [Online] 89 (12), 4163–4174. Available from: doi:10.2527/jas.2010-3703.

Chen, X. & Gomes, M. (1992) *Estimation of Microbial Protein Synthesis to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives - An Overview of the Technical Details*. Aberdeen, UK, Occasional Publication. Internasional Feed Resources Unit. Rowett Research Institute.

Conway, E.J. & Byrne, A. (1933) An absorption apparatus for the micro-determination of certain volatile substances: The micro-determination of ammonia. *Biochemical Journal*. 27 (2), 419–429.

Dai, X. & Faciola, A.P. (2019) Evaluating strategies to reduce ruminal protozoa and their impacts on nutrient utilization and animal performance in ruminants – a meta-analysis. *Frontiers in Microbiology*. [Online] 10, Available from: doi:10.3389/fmicb.2019.02648.

Eugène, M., Archimède, H. & Sauvant, D. (2004) Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livestock Production Science*. [Online] 85 (1), 81–97. Available from: doi:10.1016/S0301-6226(03)00117-9.

Favreau, A., Baumont, R., Ferreira, G., Dumont, B. & Ginane, C. (2010) Do sheep use umami and bitter tastes as cues of post-ingestive consequences when selecting their diet? *Applied Animal Behaviour Science*. [Online] 125 (3–4), 115–123. Available from: doi:10.1016/j.applanim.2010.04.007.

Guyader, J., Eugène, M., Doreau, M., Morgavi, D.P., Gérard, C. & Martin, C. (2017) Tea saponin reduced methanogenesis in vitro but increased methane yield in lactating dairy cows. *Journal of Dairy Science*. [Online] 100 (3), 1845–1855. Available from: doi:10.3168/jds.2016-11644.

Hoeltershinken, M., Plitt, U., Tammen, F.., Hoffmann, P. & Scholz, H. (1997) Influence of mouldy grass on fermentation and thiamine metabolism in bovine rumen fluid (in vitro). *Deutsche Tierarzt Wochenschrift*. 104, 17–22.

International Atomic Energy Agency (1997) *Estimation of Rumen Microbial Protein Production from Purine Derivatives in Urine. A Laboratory Manual for FAO/IAEA Co-ordinated Research Programme*. Vienna, IAEA-TECDOC-945, IAEA.

Jayanegara, A., Wina, E. & Takahashi, J. (2014) Meta-analysis on methane mitigating properties of saponin-rich sources in the Rumen: Influence of addition levels and plant sources. *Asian-Australasian Journal of Animal Sciences*. [Online] 27 (10), 1426–1435. Available from: doi:10.5713/ajas.2014.14086.

Kumar, M., Kannan, A., Bhar, R., Gulati, A., Gaurav, A. & Sharma, V.K. (2017) Nutrient intake, digestibility and performance of Gaddi kids supplemented with tea seed or tea seed saponin extract. *Asian-Australasian Journal of Animal Sciences*. [Online] 30 (4), 486–494. Available from: doi:10.5713/ajas.16.0451.

Liu, Y., Ma, T., Chen, D., Zhang, N., Si, B., Deng, K., Tu, Y. & Diao, Q. (2019) Effects of tea saponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in dorper crossbred

ewe. *Animals*. [Online] 9 (1), 29. Available from: doi:10.3390/ani9010029.

Lopes, M.G., Dominguez, J.H.E., Corrêa, M.N., Schmitt, E. & Fischer, G. (2019) Rumen-protected methionine in cattle: influences on reproduction, immune response, and productive performance. *Arquivos do Instituto Biológico*. [Online] 86, Available from: doi:10.1590/1808-1657001292018.

Low, S. (2015) Signal Grass (Brachiaria decumbens) Toxicity in grazing ruminants. *Agriculture*. [Online] 5 (4), 971–990. Available from: doi:10.3390/agriculture5040971.

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. *Livestock Science*. [Online] 129 (1–3), 56–62. Available from: doi:10.1016/j.livsci.2009.12.011.

Marrelli, M., Conforti, F., Araniti, F. & Statti, G. (2016) Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules*. [Online] 21 (10), 1404. Available from: doi:10.3390/molecules21101404.

Martínez-Aispuro, J.A., Sánchez-Torres, M.T., Mendoza-Martínez, G.D., Cordero Mora, J.L., Figueroa-Velasco, J.L., Ayala-Monter, M.A. & Crosby-Galván, M.M. (2018) Addition of calcium propionate to finishing lamb diets. *Veterinaria México OA*. [Online] 5 (4), 1–9. Available from: doi:10.22201/fmvz.24486760e.2018.4.470.

Newbold, C.J., De la Fuente, G., Belanche, A., Ramos-Morales, E. & McEwan, N.R. (2015) The role of ciliate protozoa in the rumen. *Frontiers in Microbiology*. [Online] 6, 1313. Available from: doi:10.3389/fmicb.2015.01313.

Pasaribu, T., Astuti, D., Wina, E., Sumiati & Setiyono, A. (2014) Saponin content of *sapindus rarak* pericarp affected by particle size and type of solvent, its biological activity on *Eimeria tenella oocysts*. *International Journal of Poultry Science*. [Online] 13 (6), 347–352. Available from: doi:10.3923/ijps.2014.347.352.

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. *Nutrition Research Reviews*. [Online] 22 (2), 204–219. Available from: doi:10.1017/S0954422409990163.

Ramos-Morales, E., de la Fuente, G., Duval, S., Wehrli, C., Bouillon, M., Lahmann, M., Preskett, D., Braganca, R. & Newbold, C.J. (2017) Antiprotozoal effect of saponins in the rumen can be enhanced by chemical modifications in their structure. *Frontiers in Microbiology*. [Online] 08, 1–14. Available from: doi:10.3389/fmicb.2017.00399.

Ramos-Morales, E., Lyons, L., de la Fuente, G., Braganca, R. & Newbold, C.J. (2019) Not all saponins have a greater antiprotozoal activity than their related sapogenins. *FEMS Microbiology Letters*. [Online] 366 (13), 1–7. Available from: doi:10.1093/femsle/fnz144.

Sen, A., Karim, S. & Santra, A. (2000) Effect of defaunation on carcass and meat characteristics of finisher lambs. *The Indian Journal of Animal Sciences*. 70 (6), 659–661.

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. *Journal of Dairy Science*. [Online] 74 (10), 3583–3597. Available from: doi:10.3168/jds.S0022-0302(91)78551-2.

Suharti, S., Astuti, D.A., Wina, E. & Toharmat, T. (2015) Digestibility, fermentation characteristic, protein microbial synthesis and growth performance of beef cattle fed high forage ration with lerak extract supplementation. *Pakistan Journal of Nutrition*. [Online] 14 (12), 885–891. Available from: doi:10.3923/pjn.2015.885.891.

Suharti, S., Astuti, D.A., Wina, E. & Toharmat, T. (2011) Rumen microbial population in the in vitro fermentation of different ratios of forage and concentrate in the presence of whole lerak (Sapindus Rarak) fruit extract. *Asian-Australasian Journal of Animal Sciences*. [Online] 24 (8), 1086–1091. Available from: doi:10.5713/ajas.2011.10409.

Thalib, A., Widiawati, Y. & Haryanto, B. (2010) Penggunaan complete rumen modifier (CRM) pada ternak domba yang diberi hijauan pakan berserat tinggi. *Jurnal Ilmu Ternak dan Veteriner*. [Online] 15 (2), 97–104. Available from: doi:10.14334/JITV.V15I2.647.

Wang, J.K., Ye, J.A. & Liu, J.X. (2012) Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance-a review. *Tropical Animal Health and Production*. [Online] 44 (4), 697–706. Available from: doi:10.1007/s11250-011-9960-8.

Wina, E. (2012) Saponins: Effects on rumen microbial ecosystem and metabolism in the rumen.In: Amlan, K. (ed.) *Dietary Phytochemicals and Microbes*. [Online] 9789400739260, Switzerland, Springer Netherlands, pp.311–350. Available from: doi:10.1007/978-94-007-3926-0_11.

Wina, E., Muetzel, S. & Becker, K. (2006a) Effects of daily and interval feeding of *Sapindus rarak* saponins on protozoa, rumen fermentation parameters and digestibility in sheep. *Asian-Australasian Journal of Animal Sciences*. [Online] 19 (11), 1580–1587. Available from: doi:10.5713/ajas.2006.1580.

Wina, E., Muetzel, S. & Becker, K. (2006b) The dynamics of major fibrolytic microbes and enzyme activity in the rumen in response to short- and long-term feeding of Sapindus rarak saponins. *Journal of Applied Microbiology*. [Online] 100 (1), 114–122. Available from: doi:10.1111/j.1365-2672.2005.02746.x.

Yulistiani, D., Puastuti, W., Haryanto, B., Purnomoadi, A., Kurihara, M. & Thalib, A. (2017) Complete rumen modifier supplementation in corn cob silage basal diet of lamb reduces methane emission *Indonesian Journal of Agricultural Science*. [Online] 18 (1), 33–42. Available from: doi:10.21082/ijas.v18n1.2017.p33-42 [Accessed: 23 December 2020].

Zhou, Y.Y., Mao, H.L., Jiang, F., Wang, J.K., Liu, J.X. & McSweeney, C.S. (2011) Inhibition of rumen methanogenesis by tea saponins with reference to fermentation pattern and microbial communities in Hu sheep. *Animal Feed Science and Technology*. [Online] 166–167, 93–100. Available from: doi:10.1016/j.anifeedsci.2011.04.007.