

Supplementation of *Sapindus rarak* and Garlic Extract in Feed Containing Adequate Cr, Se, and Zn on Rumen Fermentation

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ABSTRACT

The objective of the study was to evaluate the effect of *Sapindus rarak* extract (SRE) with or without garlic extract (GE) on *in vitro* ruminal fermentation. This research was conducted experimentally with a randomized block design, with 7 treatments and 5 blocks. The treatments were: R0: dairy cow feed; R1: R0 + 1.5 ppm Cr + 0.3 ppm Se + 40 ppm Zn; R2: R1 + 1.8 g/kg methanol extract of lerak fruit meal (SRE); R3: R2 + 0.25 ppm of garlic extract (GE); R4: R2 + 0.50 ppm of GE; R5: R2 + 0.75 ppm of GE; R6: R2 + 1.0 ppm of GE. The results showed that the supplementation of SRE alone or without GE did not affect the pH, however, it decreased crude fiber digestibility. The supplementations of SRE and GE, decreased crude fibre digestibility as much as 13.01% up to 16.6%. The supplementation of 1.8 g/kg SRE + 0.25 ppm GE in the dairy cattle diet was able to decrease acetate, protozoal population and increase propionate. The supplementation of 1.8 g/kg SRE and 0.25 ppm garlic represents the best combination for dairy cattle feed in improving ruminal fermentation based on feed digestibility, fermentation products, and rumen bacterial population.

Key words: *Sapindus rarak*, garlic, ruminal fermentation, rumen microbes, dairy cow

ABSTRAK

Penelitian dilakukan untuk mengkaji pengaruh suplementasi ekstrak lerak dan bawang putih terhadap fermentasi rumen dan perubahan struktur mikroba rumen secara *in vitro*. Penelitian dilaksanakan dengan menggunakan rancangan acak kelompok, dengan 7 perlakuan dan 5 blok. Adapun perlakuan yang dicobakan adalah R0: pakan sapi perah; R1: R0 + 1,5 ppm Cr + 0,3 ppm Se + 40 ppm Zn; R2: R1 + 1,8 g/kg ekstrak lerak (SRE); R3: R2 + 0,25 ppm ekstrak bawang putih; R4: R2 + 0,50 ppm dari ekstrak bawang putih; R5: R2 + 0,75 ppm dari ekstrak bawang putih; dan R6: R2 + 1,0 ppm dari ekstrak bawang putih. Hasil penelitian memperlihatkan bahwa suplementasi ekstrak lerak sendiri maupun dengan bawang putih tidak mempengaruhi pH dan pencernaan (bahan kering dan bahan organik) dan menurunkan pencernaan serat kasar sebesar 13,0% sampai dengan 16,6%. Suplementasi 1,8 g/kg ekstrak lerak dan 0,25 ppm ekstrak bawang putih menurunkan asetat, populasi protozoa, dan meningkatkan propionat. Suplementasi 1,8 g/kg ekstrak lerak dan 0,25 ppm ekstrak bawang putih merupakan kombinasi terbaik pada pakan sapi perah dalam meningkatkan fermentasi rumen dipandang dari pencernaan pakan, produk fermentasi, dan populasi bakteri rumen.

Kata kunci: *Sapindus rarak*, bawang putih, fermentasi rumen, mikroba rumen, sapi perah

INTRODUCTION

A goal of ruminant nutritionists is to manipulate the rumen microbial ecosystem to improve the efficiency of converting feed to animal products consumable by humans. During ruminal fermentation a part of consumed energy and protein are excreted (as methane and ammonia nitrogen, respectively) without utilization by

rumen microflora or host animals (Busquet *et al.*, 2006). For this reason, ruminant nutritionist has suggested optimizing diet formulation and using feed additives. In dairy cattle, the uses of antibiotics as feed additives, such as ionophore antibiotic, has been proven to be a useful tool to reduce energy (in the form of methane) and nitrogen (in the form of ammonia) losses from diet (Calsamiglia *et al.*, 2007). The use of antibiotic in feed has negative effects for human, due to the secretion of the antibiotic in milk. For this reason, scientist has recently become interested in evaluating other alternatives for manipulating gastrointestinal microflora in livestock.

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Plant extracts have been used for centuries for various purposes (traditional medicine, industrial applications, and food preservatives) because of their antimicrobial properties (Tassoul & Shaver, 2009). The use of plant extracts appears as one of the most natural alternatives to the antibiotic used in animal nutrition. Results of previous studies indicated that extract of some plants can be appropriate alternative for antibiotics growth promoters (Calsamiglia *et al.*, 2006). Saponins from fruits of *Sapindus rarak* have been reported by Wina *et al.* (2005) and Suharti *et al.* (2010) as a defaunating agent. Addition of *S. rarak* saponins *in vitro* decreased significantly protozoal counts. It is believed that the saponin-containing plants suppress methane emission by reducing protozoal population and changing the rumen fermentation pattern. The symbiotic of protozoa with methanogenic in the rumen is well established (Finlay *et al.*, 1994). However, there is only 37% of the methanogenic, that has symbiotic with protozoa, the rest live freely in the rumen ecosystem. The addition of other herbs is expected to greatly increase ruminal fermentation efficiency.

Busquet *et al.* (2005) reported that garlic oil altered fermentation by reducing the proportion of acetate and increasing the propionate in a manner similar to monensin in continuous culture. Chiquette & Benchaar (2005) showed inhibiting effect of garlic and junifer berry essential oil on the production of methane *in vitro*. Only few studies to date have investigated the combination effect of saponin from *S. rarak* and methanol garlic extract on ruminal fermentation *in vitro*. The preliminary study showed that supplementations of Cr, Se, and Zn minerals were able to increase rumen efficiency in dairy cattle; however, the methane production was still high (Prayitno & Widiyastuti, 2010). The purpose of the study was to evaluate the effects of supplementation of *S. rarak* and garlic extract in feed containing adequate Cr, Se, and Zn on rumen fermentation in order to improve fermentation efficiency.

MATERIALS AND METHODS

This research was conducted experimentally with a randomized block design, with 7 treatments and 5 blocks. The treatments were: R0: dairy cow feed (concentrate: grass, 50:50 with CP: 15.5%, TDN: 68%, NDF: 28%); R1: R0 + 1.5 ppm Cr + 0.3 ppm Se and 40 ppm Zn; R2: R1 + 1.8 g/kg of methanol extract of the lerak fruit meal (SRE); R3: R2 + 0.25 ppm of garlic extract (GE); R4: R2 + 0.50 ppm of GE; R5: R2 + 0.75 ppm of GE; R6: R2 + 1.0 ppm of GE. Mineral supplement formulated of 1.5 ppm Cr, 0.3 ppm Se, and 40 ppm Zn was based on previous study. The concentrate mix consisted of soybean meal, coconut cake meal, cassava waste, wheat pollard, molasses, dicalcium phosphate, NaCl and CaCO₃ (CP: 15.5%, TDN: 68%, NDF: 28%). King grasses were harvested and dried in the oven 65 °C over night and then milled.

In Vitro Fermentation

Rumen fluid for this experiment was collected from a non fistulated dairy cow fed a diet consisting of grass

and concentrate mixture (50:50). The rumen fluid was filtered through double layer cheesecloth. The substrate for *in vitro* rumen fermentation was a mixture of concentrate feed and dried milled king grass.

In vitro fermentation was conducted according to the method of Tilley & Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate, 40 mL McDougall buffer and 10 mL rumen fluid were added. The mixture was stirred and flushed with O₂-free carbon dioxide and the tubes were then sealed with a rubber cork with the gas release valve. All the fermentation tubes were incubated in a shaker waterbath at 39 °C for 24 h.

Preparation of Lerak Fruit (*Sapindus rarak*, SRE) and Garlic (*Allium sativum*, GE) Extract

Preparation of lerak extract was initiated by separation of seeds from the fruit. The fruits were dried in an oven at a temperature of 60 °C for 4 d, and milled. The lerak powder was macerated in methanol (1:4 w / v) overnight. The methanol was then evaporated in a rotary-evaporator. The extraction was repeated once more to produce a crude extract. The residue was then freeze dried and stored at -4 °C (adopted from Wina *et al.*, 2004; Suharti *et al.*, 2010).

Partial VFA concentration and molar proportion of VFA at 24 h of fermentation were analyzed using gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, capillary column WCOT Fused Silica 25 types mx0.32 mm, oven temperature: conditioning at 60 °C and running at 115 °C, using nitrogen as a carrier gas). Before analysis, the pH of the rumen fluid aliquot of the *in vitro* incubation was adjusted at pH 3-4 with H₂SO₄. Subsequently 1.5 mL aliquot of rumen fluid was mixed with 30 mg of sulfosalicylic acid (C₇H₆O₆S.2H₂O) and centrifuged at 12,000 rpm for 10 min (7 °C) and 0.5 µL mixed solution was injected into the gas chromatography.

Protozoal and Bacterial Counts

After 24 h incubation, 1 mL of aliquot of each treatment was taken for protozoal and bacterial counts. One mL of aliquot was mixed with 1 mL of methyl green formaldehyde (35% of formaldehyde, distilled water, methylgreen and NaCl) for protozoal count (Ogimoto & Imai, 1981). To count the number of bacterial population, the method of dilution was used at 24-hour incubation. As much as 0.05 mL of aliquot was added to the 4.95 mL diluted medium. A serial dilution 10⁻⁶, 10⁻⁷ and 10⁻⁸ were made using of Brain Heart Infusion (BHI) medium (Champod *et al.*, 2009). The count unit of bacteria in term of colony forming unit, was calculated.

Methane Production

Measurement of total gas was based on the method of Menke (1979). The CH₄ production was calculated from stoichiometry of the main VFA formed during fermentation, i.e: acetate (C2), propionate (C3), and butyrate (C4) as follows:

$$\text{CH}_4: 0.45 \text{ C}_2 - 0.275 \text{ C}_3 + 0.40 \text{ C}_4 \text{ (Moss et al., 2000)}$$

Statistical Analysis

The statistical analysis of the data was carried out according to one-way ANOVA (Steel & Torrie, 1995).

RESULTS AND DISCUSSION

The effect of supplementation of SRE with or without GE on *in vitro* ruminal fermentation characteristic is shown in Table 1 and Table 2. The supplementation of SRE alone or without GE did not affect the pH. Similar result was obtained for digestibilities of dry matter and organic matter. The supplementations of SRE and GE, decreased crude fibre digestibility as much as 13.01% up to 16.6%, whereas the supplementation of SRE alone did not decrease the crude fibre digestibility.

The supplementation of feed with Cr, Se, and Zn organic minerals was able to increase total VFA and propionate concentration and decreased total gas. Cr, Se and Zn represent essential minerals for ruminal microbes. Some studies show that the three minerals support ruminal fermentation. Cr is able to increase organic matter, NDF, and ADF digestibilities (Emami *et al.*, 2011; Sadri *et al.*, 2009). The digestibilities of OM, NDF, and ADF have positive correlation with the increase of VFA concentration. Jayanegara *et al.* (2006) informed that supplementation of organic and inorganic Cr in feed increase VFA concentration. The form of organic Cr, Se, and Zn are absorbed more efficiently relative to those of inorganic forms (Mainville *et al.*, 2009; Cope *et al.*, 2009; Panev *et al.*, 2013). Se represents an intracellular antioxidant component (GSH-Px), therefore the mineral support the activity of ruminal microbe to produce VFA. Zn is generally considered to be a stabilising agent of biological membranes. Bateman *et al.* (2004) informed that the supplementations of Zn and monensin increase the concentration of propionate and decrease the concentration of acetate.

The reduced digestibility of fiber in the treatment of ≥ 0.5 ppm garlic administration was assumed to be closely associated with alliin that affect the activity of fiber degrading bacteria. Busquet *et al.* (2005) reported that the use of garlic (315 ml/L) reduced digestibility of NDF and ADF. Similar results were also obtained for VFA concentration. However, the SRE and GE supple-

mentations significantly decreased the total gas concentration. This result was not in line with that of Yang *et al.* (2007) who reported that the supplementation of garlic powder at 5 g/d/head was able to increase the DM and OM digestibilities from 49.4% to 55.2% and 49.6% to 55.4%, respectively, however it did not affect the VFA concentration.

The supplementation of 1.8 g/kg SRE in the dairy cattle diet (R1) was able to decrease acetate, as much as 9.05%. The supplementation of *S. rarak* extracts (SRE) as much as 1.8 g/kg and 0.25 ppm GE decreased acetate concentration, as much as 5.37%. However the supplementation of 1.8 g/kg SRE + 0.5 ppm GE and 1.8 g/kg SRE + 1.0 ppm GE, increased the concentration of acetate. Increasing the molarity of acetate in the garlic addition of ≥ 0.5 ppm was assumed to be associated with alliin in stimulating the activity of the predominant Gram-positive bacteria that produce acetate. Considering that production of acetate will be followed by the release H⁺ ions, therefore the increase of acetate will have the capability of increasing methane. This is because methane is synthesized from CO₂ + H⁺ (Kebrab *et al.*, 2006).

The supplementation of 1.8 g/kg SRE (R2), 1.8 g/kg SRE+ 0.25 ppm GE (R3), 1.8 g/kg SRE + 0.5 ppm of GE (R4), 1.8 g/kg SRE + 0.75 ppm of GE (R5) for dairy cattle diet, increased propionate concentration as much as, 23.23%, 44.71%, 21.12%, and 12.14% respectively. The increased in propionate production in the present study might be attributed to a decline in protozoal numbers in the incubation media.

The treatment of the addition of 1.8 g/kg SRE (R2) and 1.8 g/kg SRE + 0.25 ppm GE (R3) effectively decreased protozoal population. The concentration of saponin from SRE (81.9%) and GE (20.13%) was able to lyse the cell membrane of protozoa. On the other side, garlic represents methane inhibitor, due to the alliin that is able to inhibit HMC-CoA reductase enzyme of the methanogenic (Busquet *et al.*, 2005). As a result, the availability of H₂ in the rumen is not used for methane synthesis, but it is used for propionate synthesis. A study by Busquet *et al.* (2005) indicated that the higher level of garlic concentration in feed increased the propionate concentration.

Table 1. Supplementation of *Sapindus rarak* extract and garlic extract on digestibility, VFA, and total gas

	Treatment						
	Control	R1	R2	R3	R4	R5	R6
pH	6.80± 0.10	6.70± 0.10	6.80± 0.10	6.80± 0.10	6.90± 0.10	6.90±0.10	6.60±0.10
IVDMD (%)	72.32± 1.50	69.18± 1.38	71.40± 0.62	68.66± 1.68	69.72± 1.92	66.06±1.79	66.24±1.45
IVOMD (%)	68.72± 1.57	64.06± 1.26	66.68± 0.59	65.28± 1.60	66.94± 2.51	63.84±1.15	63.76±1.22
IVCFD (%)	71.31± 1.05 ^a	68.25± 2.21 ^a	68.92± 2.34 ^a	62.03± 1.04 ^b	62.83± 1.37 ^b	60.61±3.17 ^b	59.47±1.96 ^b
VFA (%)	158.20±12.39 ^c	187.40±14.60 ^a	170.00±12.80 ^b	154.80±13.91 ^c	163.60±14.66 ^b	144.20±9.35 ^c	86.40±6.48 ^d
Gas production (ml)	88.20± 6.74 ^a	54.83± 6.51 ^b	21.45± 4.77 ^d	18.93± 4.37 ^b	17.57± 1.44 ^e	20.64±2.14 ^d	38.86±3.85 ^c

Note: Means in the same row with different superscript differ significantly (P<0.05). R1= dairy cow feed of BBPTU Baturraden + 1.5 ppm Cr + 0.3 ppm Se and 40 ppm Zn; R2= R1 + 1.8 g/kg of methanol extract of the flour lerak fruit (SRE); R3= R2 + 0.25 ppm of garlic extract (GE); R4= R2 + 0.50 ppm of GE; R5= R2 + 0.75 ppm of GE; R6= R2 + 1.0 ppm of GE. IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility; IVCFD= *in vitro* crude fiber digestibility.

Table 2. Supplementation of *Sapindus rarak* extract and garlic extract on VFA

	Treatment						
	Control	R1	R2	R3	R4	R5	R6
Proportion of VFA (mol/100 mol)							
Acetate	70.01±1.86 ^b	70.40±0.26 ^b	63.67±3.08 ^d	66.62±2.86 ^c	75.21±1.18 ^a	71.52±1.71 ^b	75.37±1.34 ^a
Propionate	20.17±0.06 ^{cd}	24.49±0.57 ^b	26.67±0.04 ^b	29.19±0.13 ^a	24.43±0.82 ^b	22.72±0.05 ^c	18.11±0.13 ^d
Butyrate	9.82±0.10	5.11±0.10	9.66±0.10	4.19±0.10	0.36±0.10	5.75±0.10	6.52±0.10
Acetate : Propionate (A : P)	3.47±0.13 ^b	2.87±0.24 ^d	2.39±0.16 ^d	2.28±0.15 ^d	3.08±0.20 ^c	3.15±0.07 ^c	4.16±0.02 ^a
Methane (mol/100 mol)	29.94±0.84 ^b	27.99±0.13 ^b	23.33±1.37 ^c	22.72±1.30 ^c	29.07±0.70 ^b	28.36±0.78 ^b	31.24±0.55 ^b

Note: Means in the same row with different superscript differ significantly ($P < 0.05$). R1= dairy cow feed of BBPTU Baturraden + 1.5 ppm Cr + 0.3 ppm Se and 40 ppm Zn; R2= R1 + 1.8 g/kg of methanol extract of the flour lerak fruit (SRE); R3= R2 + 0.25 ppm of garlic extract (GE); R4= R2 + 0.50 ppm of GE; R5= R2 + 0.75 ppm of GE; R6= R2 +1.0 ppm of GE.

On the other hand, result from *in vitro* study by Wallace *et al* (1994) has shown that the growth of *S. ruminantium* was not inhibited by yucca saponins, whereas growth of some other ruminal bacterial species such as *Streptococcus bovis* and *Butyrivibrio fibrisolvens* were strongly inhibited. According to Wolin & Miller (1998), *S. ruminantium* is apparently responsible for most propionate production in the rumen, and supplementation of SRE and GE in the present study might promote species such as *S. ruminantium* to fill the niche, thereby increasing the accumulation of propionate in the incubation media.

Supplementation of 1.8 g/kg SRE, and 1.8 g/kg SRE + 0.25 ppm GE, was able to decrease methane by 22.07% and 24.12%, respectively. Whereas, the other combinations did not suppress methane production. This study also showed that supplementation of 1.8 g/kg SRE, and 1.8 g/kg SRE + 0.25 ppm GE was able to decrease AP ratio from 3.47 to 2.39 and 2.28, respectively, or reduction as much as 31.22% and 34.25 %, respectively. Lila *et al.* (2005) reported that supplementation of sarsaponin in the beef diet as much as 1% of ration DM was able to decrease ruminal protozoal population and acetate from 64.0% to 60.9%, and acetate-propionate ratio changed from 3.01 to 2.61. The saponin in the *S. rarak* interacts with cholesterol of the protozoa membrane causing the protozoa cells to be lysis (Hess *et al.*, 2003). It was also reported by Sharft (2010) that the addition of saponin from Yucca up to 2.5 g/kg DM was able to decrease total gas from 57.2 ml to 3.5 ml, decreased methane from 8.3 ml to 0.3 ml and acetate from 66.94 to 0.39%. Whereas garlic powder at 150 g/kg DM decreased total gas from 57.2 ml to 0.60 ml. Suharti *et al.* (2010) reported that the

supplementation of *S. rarak* extract as much as 1.8 g/kg was able to decrease acetate from 64.8% to 60.39%, and decreased the acetate-propionate ratio from 3.01 to 2.52, and increased the total VFA from 153.4 mM to 184.6 mM. However it decreased the DM and OM digestibilities from 67.70% to 64.62% and from 66.13% to 63.65%, respectively. The study of Benchaar *et al.* (2008) showed that the dry matter, organic matter, protein, NDF, and ADF digestibilities in the diet of dairy cattle were not affected by supplementation of cinnamaldehyde extract, tannin from Quebracho, and saponin from Yucca. The digestibilities of DM and OM ranged between 62.5% to 64.3%, and 64.8% to 66.6% respectively.

Decreased CH₄ production by SRE and GE supplementation might also be a consequence of increased propionate production because propionate production indirectly competes with methanogenesis for available hydrogen. Saponins have been reported to inhibit CH₄ production *in vivo* (Santoso *et al.* 2004) and *in vitro* (Lila *et al.* 2003), which was attributed to their inhibitory effect on growth of ciliate protozoa and on cellulolytic bacteria (Benchaar *et al.*, 2008). Hess *et al.* (2003) informed that the saponin of *S. saponaria* fruit was able to decrease methane emission, in the defaunated as well as non-defaunated animals. This study showed that supplementation of SRE and 0.25 ppm GE resulted in the lowest CH₄ (Table 2), although the production of total VFA were similar (Table 1). This case indicated that the supplementation of SRE and 0.25 ppm GE resulted in higher ruminal fermentation efficiency.

The results of this study showed that the supplementation of 1.8 g/kg SRE and 0.25 ppm GE was able to decrease protozoal population in between 15.1% to

Table 3. Supplementation of *Sapindus rarak* extract and garlic extract on protozoal and bacterial populations

	Treatment						
	Control	R1	R2	R3	R4	R5	R6
Protozoa (10 ⁶ cell/ml)	16.37±2.78 ^a	9.38±2.13 ^c	9.87±3.00 ^c	13.50±2.04 ^b	13.38±3.63 ^b	14.00±2.36 ^b	8.00±3.38 ^d
Bacteria (log ₁₀ cell/ml)	9.79±0.15 ^a	8.84±0.51 ^f	9.32±0.21 ^b	9.25±0.31 ^e	9.44±0.21 ^c	9.61±0.11 ^b	9.26±0.31 ^e

Note: Means in the same row with different superscript differ significantly ($P < 0.05$). R1= dairy cow feed of BBPTU Baturraden + 1.5 ppm Cr + 0.3 ppm Se and 40 ppm Zn; R2= R1 + 1.8 g/kg of methanol extract of the flour lerak fruit (SRE); R3= R2 + 0.25 ppm of garlic extract (GE); R4= R2 + 0.50 ppm of GE; R5= R2 + 0.75 ppm of GE; R6= R2 +1.0 ppm of GE.

17.5%. Antiprotozoal effect of SRE and GE were confirmed in the present study. One possible mechanism to explain the inhibitory effect on protozoal growth is the change in the cell membrane permeability, as they form complexes with cholesterol in protozoal cell membranes and cause cell lysis (Hess *et al.*, 2003). The structure and mechanism of action of garlic extract and its main active components on rumen microbial fermentation are different from other compounds. Busquet *et al.* (2005) suggested that the antimethanogenic effect of garlic and its active components was the result of direct inhibition of Archaea microorganisms in the rumen. Archaea have unique membrane lipids that contain glycerol linked to long chain isoprenoid alcohols essential for the stability of the cell membrane (Kongmun *et al.*, 2010). Goel *et al.* (2008) reported that *Sesbania saponins* decreased methanogen population by 78% and increased *Fibrobacter succinogenes* (21%-45%) and *Ruminococcus flavofaciens* (23%-40%). Pen *et al.* (2006), observed that the inclusion of *Quillaja saponaria* extract (QSE) resulted in decrease in protozoal population by 41%, but there was no effect on methane production. The other study informed the reduction of methanogens number by reduction of protozoa, as 10%-20% of total methanogens reside in close association with protozoa (Kumar *et al.*, 2009). Ranilla *et al.* (2007) who conducted a study on the sheep rumen *in vitro* informed that the absence of ruminal protozoa in the rumen ecosystem decreased feed digestibility and methane proportion.

CONCLUSION

The supplementation of 1.8 g *S. rarak* extract and 0.25 ppm garlic extract per kilogram ration represents the best combination for dairy cattle feed containing adequate Cr, Se, and Zn minerals to improve ruminal fermentation based on feed digestibility, fermentation products, and rumen bacterial population.

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