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

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.
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 sulfoalicylic acid (C₆H₅O₂S₂H₂O) 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, methyglycine 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 10⁴, 10³ and 10² 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:

$$\text{CH}_4: 0.45 \text{C2} - 0.275 \text{C3} + 0.40 \text{C4}$$ (Moss et al., 2000)
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 supplemetations of SRE and GE, decreased crude fibre digestibility as much as 13.01% up 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 or 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 realtime 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 supplemetations 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 allicin 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-

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 | 66.66±1.79 | 66.24±1.45 |
| IVMOD (%) | 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 | 68.25±2.21 | 68.92±2.34 | 62.03±1.04 | 62.83±1.37 | 60.61±1.78 | 59.47±1.96 |
| VFA (%)   | 158.20±12.39 | 187.40±14.60 | 170.00±12.80 | 154.80±13.91 | 163.60±14.60 | 144.20±9.35 | 86.40±6.48 |
| Gas production (ml) | 88.20±6.74 | 54.83±6.51 | 21.45±4.77 | 18.93±4.73 | 17.57±1.44 | 20.64±2.14 | 38.86±3.85 |

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; IVMOD= in vitro organic matter digestibility; IVCFD= in vitro crude fiber digestibility.
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 Butyryrivibrio 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 2.52, 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 (Benchaa et al., 2008). Hess et al. (2003) informed that the saponin of S. saportaria 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 24.12% respectively.

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 | 70.0±1.86 | 70.40±0.26 | 63.67±3.08 | 66.62±2.86 | 75.21±1.18 | 71.52±1.71 | 75.37±1.34 |
| Propionate | 20.17±0.06 | 24.49±0.57 | 26.67±0.04 | 29.19±0.13 | 24.43±0.82 | 22.72±0.05 | 18.11±0.13 |
| 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 | 2.87±0.24 | 2.39±0.16 | 2.28±0.15 | 3.08±0.20 | 3.15±0.07 | 4.16±0.02 |
| Methane (mol/100 mol) | 29.94±0.84 | 27.99±0.13 | 23.33±1.37 | 22.72±1.30 | 29.07±0.70 | 28.36±0.78 | 31.24±0.55 |

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.

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

| Treatment | Control | R1 | R2 | R3 | R4 | R5 | R6 |
|-----------|---------|----|----|----|----|----|----|
| Protozoa (10⁶ cell/ml) | 16.37±2.78 | 9.38±2.13 | 9.87±3.00 | 13.50±2.04 | 13.38±3.63 | 14.00±2.36 | 8.00±3.38 |
| Bacteria (log10 cell/ml) | 9.79±0.15 | 8.84±0.51 | 9.32±0.21 | 9.25±0.31 | 9.44±0.21 | 9.61±0.11 | 9.26±0.31 |

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.

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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 flavefaciens (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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