The Efficacy of Methanol Extract of Garlic (*Allium sativum*) to Improve Rumen Fermentation Products

CH Prayitno and N Hidayat

Faculty of Animal Science, Jenderal Soedirman University
Dr. Soeparno Street No. 60, PO. Box 110, Purwokerto 53123, Central Java, Indonesia
***Corresponding author email: caribu_prayitno@yahoo.co.id**

Abstract. This study was aimed to evaluate the effect of the increase levels of garlic extract (*Allium sativum, As*) in beef cattle feed on rumen fermentation products and microbial populations. The materials used were rumen fluid and control feed (40 % fermented rice straw:60% concentrate). The research method was experimental using Randomized Complete Block Design (RCBD) applying 7 treatments which were repeated 3 times. The tested feed treatments were S0 (control feed), S1 (S0 + 1.5 ppm of Cr, S2 (S0 + 250 ppm of As extract), S3 (S1 + 250 ppm of As extract), S4 (S1 + 500 ppm of As extract), S5 (S1 + 750 ppm of As extract) and, S6 (S1 + 1000 ppm of As extract). The data were analyzed using analysis of variance and followed by Honestly Significant Difference test. The results showed that the treatment effects on total bacteria, protozoa population, total gas and methane were highly significant but had no effect on dry matter digestibilityDMD and organic matter digestibility OMD and production of VFA. The treatments increased total bacteria but decreased total gas and methane. The best result was achieved on treatment S6 (supplementation of As at the level of 1000 ppm in the feed) of which the feed with adequate Cr will result in the most efficient of fermentation.

Keywords: Extract of *Allium sativum*, organic Cr, microbial population, rumen fermentation, beef cattle

Abstrak. Penelitian ini bertujuan untuk mengevaluasi pengaruh dan level penambahan ekstrak bawang putih (*Allium sativum*, As) dalam pakan sapi potong terhadap produk fermentasi dan populasi mikroba rumen. Materi yang digunakan dalam penelitian adalah cairan rumen sapi potong dan pakan kontrol (jerami fermentasi : konsentrat; 40:60%). Metode penelitian adalah eksperimental menggunakan Rancangan Acak Kelompok (RAK) 7 perlakuan, setiap perlakuan diulang 3 kali. Pakan perlakuan yang diujicobakan pada sapi potong yaitu S0: pakan kontrol, S1: S0 + 1,5 ppm Cr, S2: S0 + 250 ppm ekstrak As, S3: S1 + 250 ppm ekstrak As, S4: S1 + 500 ppm ekstrak As, S5: S1 + 750 ppm ekstrak As, S6: S1 + 1000 ppm ekstrak As. Data dianalisis menggunakan analisis variansi dilanjutkan uji Beda Nyata Jujur. Hasil penelitian menunjukkan perlakuan berpengaruh terhadap total bakteri, populasi protozoa, gas total dan metan tetapi tidak berpengaruh terhadap kecernaan bahan kering dan bahan organik serta produksi VFA. Perlakuan mampu meningkatkan total bakteri serta menurunkan gas total dan metan. Hasil terbaik, pada pakan perlakuan S6 yaitu suplementasi ekstrak As pada taraf 1000 ppm pada pakan yang tercukupi Cr organik menghasilkan efisiensi fermentasi yang paling baik.

Kata kunci : Ekstrak *Allium sativum*, mineral mikro organik, populasi mikroba, fermentasi rumen, sapi potong

Introduction

Increasing beef cattle population is one of the main focuses of Indonesia agriculture sector. The low productivity of beef cattle is affected by the feed factor. Low quality of forage does not directly reduce productivity. On the other hand, the good feed is not necessarily utilized optimally by livestock, it is partly due to microbial fermentation of feed in the rumen. In low-quality feed, protozoa prey bacteria as a source of nitrogen. This condition is not optimum for rumen fermentation, mainly due to the reduced population of fiber-degrading bacteria. Fermentation of feed will produce energy, methane and CO_2 (Hungate, 1988) which causes a decrease in the availability of minerals. One step in optimizing feed utilization can be achieved through mineral adequacy, defaunation of protozoa, and suppression of

methane formation. Selenium, chromium and zinc in the diet can increase rumen fermentation (Prayitno and Widiyastuti, 2010) and garlic can be used as a supplement for methane inhibition. Newbold et al. (1995), reported only about 37% of methanogen is associated with protozoa. This means that when only the protozoa is eliminated, it reduces methanogen population as much as 37% and the the other 63% of methanogenic will remain to produce methane. This approach is therefore absolutely necessary to suppress the methanogens activity. Hart et al. (2006) reported that *Allium sativum* (garlic) via the action of allicin has the ability to reduce methane production which directly lowers methanogens, and does not affect the bacterial population in the Rusitec fermenter. Antimethanogenic activity of garlic can affect the stability of the cell membrane of Archea (methanogens) that contains isoprenoid alcohol. Archea methanogen isoprenoid synthesis is catalyzed by HMG-CoA (Hidroxymethylglutaryl-CoA) reductase, and allicin of garlic has strong ability to inhibit HMG-CoA reductase, thus the methanogenesis is inhibited. This research was aimed to evaluate the effect of the addition of of *Allium sativum* extract levels in beef cattle feed on the rumen fermentation products and microbial populations.

Materials and Methods

The materials used in this study were beef cattle rumen fluid and control feed (40% fermented rice straw: 60% concentrate), organic Chromium (Cr), methanol extract of garlic (*Allium sativum*) without husk and materials for in-vitro analysis. Concentrate contained cassava, pollard, coconut meal, corn meal, mineral, salt and urea. The first step, the garlic purchased from traditional markets was peeled off, finely crosscut, oven-dried at 70^0C for 72 hours then ground by a waring blender to turn it into garlic powder. The garlic powder was mixed with methanol (1:4) then macerated for 12 hours. The extract was obtained by putting the macerated mixture into rotaryevaporator, then oven-heated at 60^0C for 4 days to get the dry garlic extract (that was ready to use).

Fermented rice straw was prepared by chopping it into 15 cm long of dry rice straw (Dry Matter content of 85%). The EM4 as source of microorganism was activated by means of adding 0.2% of EM4 plus 2% of fine bran and molasses which were then put into 1 l of aquadest. The mixture was concealed free of air and left for 24 hours. The chopped rice straw was sprayed with the activated EM4, then the mixture was put into plastic bags for 14 days of incubation. After fermentation, rice straw was dried at 70^0 C for 3 days, then finely ground.

The research method was an in vitro experimental. There were 7 treatments and 3 replications in thefollowing treatments: S0: control feed (CP: 14.64%, CF: 21.14%, TDN: 64,5%); S1: S0 + 1.5 ppm organic Cr; S2: S0 + 250 ppm extract of *Allium sativum*; S3: S1 + 250 ppm extract of *Allium sativum*; S4: S1 + 500 ppm extract of *Allium sativum*; S5: S1 + 750 ppm extract of *Allium sativum*; S6: S1 + 1000 ppm extract of *Allium sativum*.

The variables observed in this study were (1) dry matter digestibility (DMD) (2) organic matter digestibility (OMD) (3) total VFA concentration, (4) protozoa population, (5) total bacteria, (6) total gas production and methane. In vitro technique applied was that of Tilley and Terry (1963). Total VFA concentration measurements were carried out according to Departement of Dairy Science *(*1966).

Rumen protozoa population was calculated based on the staining technique using methylgreen formalin saline solution (MFS) in physiological saline solution and then fixed with formalin (Ogimoto and Imai, 1981). (1) Stages of enumeration (the supernatant was diluted 5

times with a solution of MFS in the ratio of 1:1:3 (rumen fluid : MFS : distilled water)); (2) Mixture was pipetted 1-2 drops, then placed in a counting chamber with a thickness of 0.1 - 0.2 mm; (3) Protozoa colonies were counted in 5 squares; (4) Protozoa population was calculated using the following formula: protozoa population = 100 x number of colonies x dilution factor. The calculation of total rumen bacteria followed the procedure of Suryahadi (1990) and the measurement of total gas was based on the method of Menke (1979).

Results and Discussion

Dry matter digestibility (DMD) and organic matter digestibility (OMD). The average values of all the treatments of rumen fermentation products and microbial populations in beef cattle trial were listed in Table 1. The results of this study showed that the supplementation by using As extract into the fermented-rice straw basal feed that was enriched with Cr had no significant effect on DMD and OMD. These results confirmed that the active substances of garlic (allicin and saponin) were not able to stimulate cellulolytic bacterial activity. 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 cinnamaldehide 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. 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 digestibities from 49.4% to 55.2% and 49.6% to 55.4%, respectively, however it did not affect the VFA concentration.

Total VFA concentration. Supplementation of *Allium sativum* to 1000 ppm in the feed of beef cattle which is adequite in Cr was unable to influence the increase in VFA concentration (Table 1). The condition was likely due to suboptimum improvement of DMD and OMD, and constant populations of protozoa in cattle that resulted in fiber-degrading bacteria population remained stable, therefore, the energy in the form of VFA did not differ. Yang et al. (2007) and Busquet et al. (2005) reported that the addition of *Allium sativum* in the feed of dairy cows showed different results compared to the control concerning the concentration of total VFA. Similar results were reported by Cardozo et al., (2004) that the administration of 7.5 mg/kg DM of *Allium sativum* (0.7% allicin) in the feed of dairy cows in fistula showed similar total VFA concentrations relative to controls.

Population of protozoa. Supplementation of As extract up to 1000 ppm highly significantly affects the protozoa population (Table 2). The supplementation of As extract into fermented-rice straw feed that was enriched with Cr up to the level of 1000 ppm tended to decrease protozoa population. At the level of 250 ppm and 1000 ppm, the supplementation of As was effective to decrease protozoa population. The decrease of protozoa of 42 percent decreased methane concentration, because 15-37% of methanogens work symbiotically with protozoa (Newbold et al., 1995). The results of this study illustrated that the 250 ppm and 1000 ppm concentration of As extract had the ability to decrease protozoa population (defaunating agent). Kongmun et al. (2011) reported that the content of As saponin as much as 20.94% was able to function as a defaunating agent.

Total bacteria. The results (Table 2) showed that the addition of garlic powder affected bacteria populations ($P<0.01$). Micro mineral (Cr) supplementation and *Allium sativum* in feed could increase total bacteria. This showed that allicin from *Allium sativum* was capable of stimulating the growth of certain types of bacteria.

Table 1. The Effect of *Allium sativum* extract supplementation in feed of beef catle on rumen fermentation products

Parameter	Treatments						
	S0	S1	S2	S3	S4	S5	S6
DMD (%)	54.15[a]	52.47[a]	55.55[a]	54.63[a]	54.49[a]	54.83[a]	54.07[a]
OMD (%)	44.04[a]	42.36[a]	45.22[a]	44.00[a]	43.16[a]	44.50[a]	43.68[a]
VFA (mM)	133.17[a]	134.17[a]	153.83[a]	129.17[a]	150.50[a]	137.83[a]	157.17[a]
Gas total /ml	9.72[a]	6.84[ab]	8.64[a]	4.68[bc]	4.68[bc]	3.60[bc]	1.80[c]
Methane/ml	2.33[a]	1.64[ab]	2.07[a]	1.12[bc]	1.12[bc]	0.86[bc]	0.43[c]

Values bearing different superscripts at the same rows differ significantly (P<0.05)
S0: control feed, S1: S0 + 1.5 ppm Cr, S2: S0 + 250 ppm As, S3: S1 + 250 ppm As, S4: S1 + 500 ppm As, S5: S1 + 750 ppm As, S6: S1 + 1000 ppm As

Table 2. The effect of *Allium sativum* extract supplementation in feed of beef catle on rumen microbial population

Parameter	Treatments						
	S0	S1	S2	S3	S4	S5	S6
Protozoa (10^6)	13.33[ab]	12.00[ab]	14.17[ab]	7.00[c]	11.92[b]	15.33[ab]	8.33[c]
Total Bacteria (log 10)	9.41[c]	9.83[ab]	9.69[bc]	9.47[c]	9.95[ab]	9.74[ab]	9.96[a]

Values bearing different superscripts at the same rows differ significantly (P<0.05)
S0: control feed, S1: S0 + 1.5 ppm Cr, S2: S0 + 250 ppm As, S3: S1 + 250 ppm As, S4: S1 + 500 ppm As, S5: S1 + 750 ppm As , S6: S1 + 1000 ppm As.

The increase of As extract increased total bacterial count and decreased total gas concentration as well as methane gas concentration. This case indicated that the supplementation of As extract decreased or inhibited the growth of fiber-degrading bacteria (side product as H2) and methanogen. The decrease in H2 concentration inhibited the methanogens to synthesize methan, therefore, the fermentation process would be more efficient; to synthesize 1 mol of methane, 130 KJ/mol of energy is needed (Sahakian et al., 2009). Kebreab et al. (2006) stated that the fermentation process in the rumen involved 3 reactions, namely (1) fermentation reaction that produces H2, for example acetate, butyrate, and for growth of microorganisms that use amino acids, (2) fermentation reaction that uses H2, for example propionic acid production, and other, VFAs and the growth of microorganisms that use NH3, (3) the excessive productions of H2 and CO2 produce CH4 by methanogenic bacteria. The results showed that the addition of garlic powder affects bacterial populations (P<0.01). Goel et al. (2008) reported that *Sesbania saponins* decreased methanogen population by 78% and increased *Fibrobacter succinogenes* (21%-45%) and *Ruminococcus flavefaciens (*23%-40%).

Total gas production. The results (Table 2) showed that the supplementation of *As* in cattle feed lowered total gas production (P<0.01). It indicated that the supplementation of *As* in an adequate mineral feed can decrease the total gas production in beef cattle. The decrease in total occured in the treatment combination of mineral supplementation with 250-1000 ppm *As* extracts (S3-S6). The decline ranged from 51.8% - 81.5% relative to the control (S3-S6 vs. S0; 4.68 to 1.8 vs. 9.72 (P<0.01)). The condition was likely due to the influence of *As* in inhibiting the growth of methanogenic bacteria and methane in the rumen of beef cattle. The decrease in total gas resulted in efficient fermentation of feed to energy through minimizing the energy loss.

Methane is synthesized from $CO_2 + H^+$ (Kebreab et al*.,* 2006). Sahakian et al*.* (2009) and Suharti et al. (2009) stated that the formation of methane in the rumen by methanogenic caused energy loss up to 15% of the total ingested chemicals energy. *Allium sativum* effective in lowering total gas was also reported by Staerfl et al., (2010) that supplementation of 150 g/kg DM of *As* in cow feed could reduce the methane amount 4.18 ml 8.35 ml of control and lower total gas by 73,1 ml/l of control 145.6 ml/l. Kongmun et al*.,* (2010) added of 4 mg *As* and 8 mg coconut oil in the diet, and they were able toreduce methane as much as 18.9% relative to controls and 45.5 mol lower total gas by 13.7% from 59.8 ml during the measurement of 72 hours.

Methane. Results on Table 1 showed that garlic powder was significant in reducing methane gas or inhibiting the occurrence of methanogenesis. Although the more garlic powder given the lower methane gas became, the optimum level of each animal differed due to different types of feed and rumen microbial population. The results of Busquet et al. (2005) showed that the addition of garlic oil at 3000 mg/L reduced total VFA and optimum dosage in dairy cows was at 312 mg/L. The use of garlic oil at 312 mg/L decreased acetate, increased propionate and valerate and lowered ratio of C2: C3 from 3.2 to 1.4. Kim et al*.* (2012) informed that garlic extracts by in vitro was more effective in reducing methane (CH4) than onion (onion), and ginger. Garlic also lowered the ratio between the acetate:propionate (A:P). Emissions of methane in ruminal fermentation are exclusively connected with the ratio A:P. The decline in methane emissions will increase the concentration of propionic acid; therefore, the ratio A:P is low (Sutardi, 1977; Mitsumori et al., 2008). Kongmun et al*.* (2011) who supplemented garlic powder at 100 g/per day, obtained the results of the increased propionate and decreased acetate. Annasori et al*.* (2012) added that in vitro using 5 mg of garlic powder on the 96-hour incubation time effectively lowered total gas compared to the control. The structure and action mechanism of garlic extract and its main active components on rumen microbial fermentaion are different from those of other compounds. Busquet et al*.* (2005) suggested that the antimethanogenic effect of garlic and its active components were the result of direct inhibition of Archaea microorganisms in the rumen. Archaea microorganisms have unique membrane lipids that contain glycerol linked to long chain isoprenoid alcohols which are essential for the stability of the cell membrane (Kongmun et al*.,* 2010). Archea methanogen isoprenoid synthesis is catalyzed by HMG-CoA (Hidroxymethylglutaryl-CoA) reductase. Allicin of Garlic has strong ability in inhibiting HMG-CoA and methanogenesis. Clark et al*.* (2011) stated that several factors could affect methane production, including feed quality, type of forage and feed additives. The results of this study were consistent with the results of Patra et al*.* (2006), Bodas et al. (2009), Sirohi et al. (2009), Bunglawan et al*.* (2010) and Kholif et al. (2012).

Conclusions

Supplementation of organic micro minerals and extracts of *Allium sativum* either separately or in combination, is more effective and significant to increase microbial population and rumen fermentation products. Supplementation of 1000 ppm of *Allium sativum* extract is effective in cattle feed that contain adequate Cr to improve the efficiency of rumen fermentation.

References

Anassori E, B Dalir-Naghadeh, R Pirmohammadi, A Taghizadeh, S Asri-Rezaei, S Farahmand-Azar, M Besharati and M Tahmoozi. 2012. In vitro assesment on digestibility of forage based sheep diet, supplemented with raw garlic, garlic oil and monensin. Veterinary Res. Forum. 3:5-11.

Benchaar C, TA MacAllister and PY Chouinard. 2008. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cow fed cinnamaldehyde, quebracho condensed tannin or *Yucca schidigera* saponin extracts. J. Dairy Sci. 91:4777:4786.

Bodas R, S Lopez, M Fernandez, R Garcia-Gonzales, R J Walace and J S Gonzalez. 2009. Phytogenic additives to decrease in vitro ruminal methanogenesis. Options Mediterraneennes. 85:279-283.

Bunglawan SJ, C Valli, M Ramachandran and V Balakrishnan. 2010. Effect of supplementation of herbal extracts on metahogenesis in ruminants. Livestock Res.RurDev. 22. 5-15.

Bryden WL and EF Annison. 1998. Prespectives on Ruminant Nutrition and Metabolism. Department of Animal Science, University of Sydney, Camden N. S. W. 2570, Australia.

Busquet M, S Calsamiglia, A Ferret, W Cardozo and C Kamel. 2005. Effect of Cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continous culture. J. Dairy Sci. 88:2508-2516.

Cardozo PW, S Calsamiglia, A Ferret and C Kamel. 2004. Effects of Natural plant extracts on ruminal protein degradation and fermentation profiles in continous culture. J. Anim. Sci. 82:3230-3236.

Departement of Dairy Science. 1966. General Laboratory Procedures. University of Wisconsin, USA. Pp. 36-70.

Goel G, HPS Makkar and K Becker. 2008. Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. J. Appl. Microbiol. 105:770-777.

Hart KJ, SE Girwood, S Taylor, DR Yanez-Ruiz and CJ Newbold. 2006. Effect of allicin on fermentation and microbial populations in the rumen simulating fermentor. Reproduction Nutrition Development. 46(supplement):97-115.

Hungate RE. 1988. The Rumen and Its Microbes. Applied Science. Academic Press. New York.

Kebreab E, K Clarck, Wagner-Riddle and J France. 2006. Methane and nitrous oxide emissions from Canadian agriculture. A review. Can. J. Anim. Sci. 86:135-158.

Kholif SM, TA Morsy, MM Abdo, OH Matloup and AA Abu El-Elia. 2012. Effect of supplementating lactating goats rations with garlic, cinnamon or ginger oils on milk yield, milk composition and milk fatty acids profile. J. Life Sci. 4:27-34.

Kim ET, CH.Kim, KS Min and SS Lee. 2012. Effect of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro. Asian-Aust. J. Anim. Sci. 25:806-811.

Kongmun P, M Wanapat, P Pakdee and C Navanukraw. 2010. Effect of coconut oil and garlic powder on in vitro fermentation using gas production technique. Livestock Sci. 127:38- 44.

Kongmun P, M Wanapat, P Pakdee, C Navanukraw and Z Yu. 2011. Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livestock Sci. 135:84-92.

Mitsumori M and W Sun. 2008. Control of rumen microbial fermentation for mitigrating methane emissions from the rumen. Asian-Aust. J. Anim. Sci. 21:144-154.

Newbold CJ, B Lassalas and JP Jouay. 1995. The Importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Letters in App. and Micro. 4:230-234.

Ogimoto K and S Imai. 1981. Atlas of Rumen Microbiology. Japan Science. Societes Press. Tokyo.

Patra AK, DN Kamra and N Agarwal. 2006. Effect of plant extracts on in vitro methanogenesis enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim. Feed Sci. and Tech. 128:276-291.

Prayitno CH and T Widiyastuti. 2010. The study on Selenomethionin, Yeast Chromium and Zinc Proteinat on Dairy Cattle Feed (Overview of the In vitro). Proceedings of the National Seminar: Agribusiness Livestock Development Perspective. Animal Science Faculty of UNSOED. Purwokerto.

Sahakian AS , Sam-Ryong Jee and M Pimentel. 2009. Methane and the gastrointestinal tract. Review. Dig Dis. Sci. Springer.

Sirohi SK, N Pandey, N Goel, B Singh, M Mohini, P Pandey and PP Chaudhry. 2009. Microbial activity and ruminal methanogenesis as affected by plant secondery metabolites in different plant extracts. Int. J. of Civ. and Env. Eng. 1:1

Staerfl SM, M Kreuzer and CR Soliva. 2010. In vitro screening of unconventional feeds and various natural supplements for their ruminal mathane mitigation potential when included in a maize-silage based diet. J. Anim. and Feed Sci. 19:651-664.

Suharti S, A Astuti and E Wina. 2009. Nutrient digestibility and performance studies of Peranakan Ongole (PO) were given flour lerak (*Sapindus rarak*) in the ration. JITV 14:200-2007.

Suryahadi. 1990. Ruminant Nutrition. Inter-University Centre of Biological Sciences. Bogor Agricultural University.

Sutardi T. 1977. Overview Ruminologi. Course materials Dairy Cattle Ranch. Wood Ambon Lembang. Directorate-General of Livestock.FAO, Bandung.

Tilley JMA and RA Terry. 1963. A two stage technique for the in vitro digestion of forage crops. J. British Grassland Soc. 18:104-111.

Wanapat M, P Khejornsart, P Pakdee and S Wanapat. 2008. Effect of supplementation of garlic powder on rumen ecology and digestibility of nutrient in ruminant. J. Sci. Food and Agri. 88:2231-2237.

Yang W, C Benchaar, BN Arnetaj, AV Chaves, ML He and TA McAllister. 2007. Effects of garlic and junifer berry essential oils on ruminal fermentation and on the site and extend of digestion in lactating cows. J. Dairy Sci. 90:5671-5681.