

Effect of Supplementation of Garlic Husk Extract to The Feed of Dairy Goats on The Fermentation Product and Ruminal Microbe

Yusuf Subagyo dan Caribu Hadi Prayitno

Faculty of Animal Science, Jenderal Soedirman University,
Jl. Dr. Suparno No. 60, Purwokerto 53123, Central Java, Indonesia
*Corresponding author email: subagyo_yusuf@hotmail.com

Abstract. This study was designed to examine the effects of supplementation with husk extract of garlic (*Allium sativum*) in the feed of dairy goats containing sufficient amount of organic minerals (Selenium, Chromium and Zinc) on the fermentation and microbes in the rumen. The materials used in this study were the rumen fluid of goat, goat ration composed of 60% grass, 35% tofu, and 5% concentrate (CP 11.90%, CF 28.57%, 60.94% TDN). The research method was experimental using a completely randomized design (CRD). The treatments were tested, namely R0: control diet; R1: R0 + Cr + 1.5 ppm 40 ppm Zn lysinat + 0.3 ppm Se; R2: R1 + 15 ppm of garlic husk extract (*Allium sativum*); R3: R1 + 30 ppm garlic husk extracts ; R4: R1 + 45 ppm of garlic husk extrat; and R5: R1 + 60 ppm garlic husk extract. The results showed that the treatment effect on a decrease in dry matter (DMD) and organic matter digestibility (OMD), protozoa and total gas in total, however, there was an increase in total VFA concentrations. The treatment gave a linear response to the DMD, ie $Y = 50.412 - 0.1651X$ and OMD, $Y = -0.1768X + 50.319$. However, in response VFA is cubic, with a line equation $Y = 203.16 - 3.2646X + 0.2447X^2 - 0.0033X^3$. It could be concluded that supplementation of garlic husk extract and organic minerals can improve rumen fermentation with the best level at 25 ppm.

Key words: Garlic husk extract, micro minerals, rumen fermentation, dairy goat

Abstrak. Penelitian ini dirancang untuk mengkaji pengaruh suplementasi ekstrak kulit bawang putih (*Allium sativum*) dalam pakan kambing perah yang tercukupi mineral organik (Selenium, Chromium dan Seng) terhadap hasil fermentasi dan mikroba pada rumen. Materi yang digunakan adalah cairan rumen kambing, ransum kambing yang tersusun atas 60% rumput gajah, 35% ampas tahu dan 5% konsentrat (PK 11,90%, SK 28,57%, TDN 60,94%). Metode penelitian adalah eksperimental menggunakan Rancangan Acak Lengkap (RAL). Perlakuan yang diujicobakan yaitu R0: pakan kontrol; R1: R0 + 1,5 ppm Cr + 40 ppm Zn lysinat + 0,3 ppm Se; R2: R1 + 15 ppm ekstrak kulit bawang putih (*Allium sativum*); R3: R1 + 30 ppm ekstrak kulit bawang putih ; R4: R1 + 45 ppm ekstrak kulit bawang putih ; dan R5: R1 + 60 ppm ekstrak kulit bawang putih . Hasil penelitian menunjukkan bahwa perlakuan berpengaruh terhadap penurunan pencernaan bahan kering (KBK) dan pencernaan bahan organik (KBO), protozoa dan gas total, namun demikian terjadi peningkatan konsentrasi VFA total. Perlakuan memberikan respon linier terhadap Kecernaan Bahan Kering, yaitu $Y = 50.412 - 0,1651X$ dan KBO, $Y = -0,1768X + 50,319$. Namun demikian pada VFA responnya adalah kubik, dengan persamaan garis $Y = 203,16 - 3,2646X + 0,2447X^2 - 0,0033X^3$. Dapat disimpulkan bahwa suplementasi ekstrak kulit bawang putih dan mineral organik dapat memperbaiki fermentasi rumen dengan level terbaik 25 ppm.

Kata kunci : ekstrak kulit bawang putih, mineral mikro, fermentasi rumen

Introduction

Rumen fermentation process is highly dependent on the activity of rumen microbes

in low quality feed (high fiber) and protozoa as one of the rumen microbes that will use bacteria as a source of nitrogen. This condition

leads to low productivity of livestock. Naturally, the process of fermentation in the rumen in addition to generate Volatile Fatty Acids (VFA) it also produces methane gas. The formation of methane gas leads to a decrease in the efficiency of rumen fermentation, because approximately 15% of the total ingested energy is used for the synthesis of methane. The role of methanogens to produce methane gas is strongly influenced by the condition of feed and feed additives. Approximately 35% of methanogenesis is in symbiotic relationship with protozoa.

Garlic (*Allium sativum*) has been known to have Allicin and organosulfur that can be used as an antibacterial by inhibiting the growth of free living methanogens. *Allium sativum* organosulfur is capable of inhibiting the enzyme HMG-CoA because methanogens lipid membrane is not formed. Garlic husk contains seven times more polyphenols than the garlic bulk (Kim et al., 2009). Allicin is included in the class of polyphenols that can reduce the population of methanogens (Prayitno et al., 2013; Wanapat et al., 2013).

The use of chromium minerals in the feed will cause fermentation pattern into the direction of glucogenic Based on the best research results of organic mineral supplementation, Prayitno and Widiyastuti (2010) reported that supplementation of dairy cattle feed with 0.3 ppm of Se + 1.5 ppm of Cr + 40 ppm of Zn proteinate effectively improved feed efficiency. A study was conducted to evaluate the effect of the level of extract husk of garlic (*Allium sativum*) in dairy goats diets that were adequated in Selenium, Chromium and Zinc-Lysinat on rumen fermentation and rumen microbial structures by in vitro.

Materials and Methods

The materials used in this study were a complete feed of dairy goats in the form of a mixture of elephant grass, concentrates and

tofu with a ratio of 60, 35, and 5%, rumen fluid of dairy goats, herbal extracts such as garlic husk extract (*Allium sativum*), organic minerals such as Cr, Se, and Zn proteinate.

The research was conducted at the Laboratory of Feedstuff, Faculty of Animal Science, Jenderal Soedirman University, Purwokerto. The experiment was subjected to a completely randomized design (CRD) with 6 treatments and 4 replications. The parameters measured in the study were (1) dry matter digestibility (DMD), (2) organic matter digestibility (OMD) using the method of Tilley and Terry (1963), and (3) the total concentration of Volatile Fatty Acid (VFA) by steam distillation method. Total gas was administered to the methods of Menke et al. (1979) and protozoa count use was according to Ogimoto and Imai methods (1982). Data were analyzed by using SPSS 20.

The treatments were R0: control feed of dairy goats control (11.90% CP, 28.57% CF, 60.94% TDN); R1: R0 + organic minerals (0.3 ppm of Selenium + 1.5 ppm of Chromium + 40 ppm of Zinc-proteinate) + 0 ppm garlic husk extract; R2: R0 + organic mineral + 15 ppm of garlic husk extracts; R3: R0 + organic mineral + 30 ppm of garlic husk extracts; R4: R0 + organic mineral + 45 ppm of garlic husk extracts; and R5: R0 + organic mineral + 60 ppm of garlic husk extracts.

Results and Discussion

Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

The results showed that garlic husk extract supplementation and organic minerals highly significant decrease ($P < 0.01$) on a dry matter digestibility (DMD) and organic matter digestibility (OMD) (Table 1).

The DMD and OMD average values ranged from 40.93 to 53.17% and 40.09 to 53.16%, respectively, with the highest DMD and OMD values in control diet ($53.17 \pm 2.89\%$ and

Table 1. Effect of supplementation of garlic husk extract on DM, OMD, and VFA

Treatments	DMD (%)	OMD (%)	VFA (mM)
R0	53.17±2.89 ^b	53.16±2.84 ^b	204.00±24.71 ^b
R1	45.66±0.91 ^a	45.37±1.18 ^a	194.67±20.23 ^b
R2	43.88±1.69 ^a	43.29±1.47 ^a	240.67±29.48 ^c
R3	41.94±2.09 ^a	41.31±1.89 ^a	245.33±11.72 ^c
R4	42.64±2.93 ^a	41.93±2.85 ^a	170.67±12.06 ^a
R5	40.93±2.58 ^a	40.09±2.85 ^a	174.67±14.47 ^a

R0: control feed, R1: R0 + Cr + 1.5 ppm 40 ppm Zn lysinate + 0.3 ppm Se, R2: R1 + 15 ppm garlic husk extract (*Allium sativum*), R3: R1 + 30 ppm garlic husk extract, R4: R1 + 45 ppm of garlic husk extract, R5: R1 + 60 ppm of garlic husk extract

53.16±2.84%). This higher value than the results demonstrated that the garlic husk extract in the diet of dairy goats contained sufficient amount of organic minerals (Chromium, Selenium, and Zn-Lysinat) that suppressed the activity of rumen microbes.

The data of Table 1 showed the lack of response and DMD decreased in feed treatment. The decreased feed DMD and OMD allegedly caused a decrease in fiber degrading bacteria, whereas 60% of the feed consisted of fibrous feed. This decrease was probably due to a substance suspected of allicin and tannin content of 7.97% methanol extracted *Allium sativum*. Tannins can bind to protein feed, resulting in a difficult protein degradability by rumen microbes leading to a decrease of feed digestibility. The decreased DMD and OMD could also result from the content of 20.94% saponin in *Allium sativum* (Sirohi et al., 2009)

Further test results using orthogonal polynomials provided a linear response to the DMD and OMD with line equation $Y = -0.1651X + 50.412$ ($R^2 = 97.54\%$) for the DMD and $Y = -0.1768X + 50.319$ for OMD ($R^2 = 75.93\%$).

Concentration of Total Volatile Fatty Acids

Effect of treatment to Concentration of Total Volatile Fatty Acids (VFA) is presented in Table 1. The mean concentration of total VFA ranged from 170.67 to 245.33 mM, exceeded the VFA adequate value of 80-160 mM (Sutardi, 1983)) for rumen microbial growth. It showed that garlic husk extract with sufficient organic minerals (Chromium, Selenium, and Zn-

proteinate) in the diet of dairy goats, influenced the total VFA concentration. The research data shows that the increase in the concentration of total VFA is in response to feed R2 and R3 feed.

An increase in the total production of VFA showed that an increase in rumen microbial activity, although lowering DMD and OMD. This is because the contents of the VFA is the result of the activity of the bacteria during fermentation in the rumen.

Jayanegara et al. (2006) reported that the VFA production in organic Cr-supplemented ration was likely more stable than inorganic Cr-supplemented diets with increasing levels of Cr. Cr supplementation, either inorganic or organic produced total VFA production in the range of optimum and feasible level for the survival of ruminants, was between 80-160 mM. Karsli and Russell (2001) also stated that the supply of micro-nutrients, especially minerals was the determinant of the effectiveness of rumen microbial protein synthesis. Minerals served importantly in supporting the efficiency of microbial protein synthesis in which minerals were the activator and components of microbial enzymes.

Kongmun et al. (2010) reported that garlic (*Allium sativum*) had high crude protein content up to 19.2%. Protein as the basic ingredient helped in the process of preparing the VFA. Satter and Slyter (1974) stated that the VFA production as a source of carbon skeleton did not only derive from carbohydrates, proteins could also contribute carbon skeletons to form

VFA and energy to the body of livestock. This is because the protein content of the feed would be degraded into amino acids which would subsequently undergo deamination into NH_3 and α keto acid. A keto acid was converted to VFA, in the form of iso-butyric, iso-valerate and 2 methyl butyrate that were used as the carbon framework for rumen microbial protein synthesis. This was in accordance to Sutardi et al. (1983), that the α keto acid is converted into VFA (iso butyrate, iso-valerate and 2 methyl butyrate) used as the carbon framework for rumen microbial protein synthesis.

Garlic husk extract supplementation in the level of 45 ppm (R4) and 60 ppm (R5) in diet with sufficient amount of organic mineral (1.5 ppm Cr + 40 ppm Zn lysinat + 0.3 ppm Se) decreased total VFA production, this was because the level of the garlic husk extract supplementation in feed was considered too high for favourable influence on the rate of carbohydrate and protein degradation in the rumen, thereby resulted in uncoupling reaction. Russel et al. (1992) stated that the degradation rate of carbohydrates and protein feed in the rumen may have a considerable influence on the final product of fermentation (VFA). Consequently, differences in the degradation rate depended on the degree of synchronization and comparative degradation of protein in the rumen. If the N degraded faster than other sources of energy (carbohydrates), then the product ammonia N degradation would be transferred to the liver, and then the fraction was recycled into the digestive tract and was mostly excreted with urine. Conversely, if the amount of energy available was beyond the availability of N, the growth of microbes and rumen fermentation efficiency decreased. This was partly due to uncoupling fermentation, namely energy (ATP) was used not for protein synthesis, but rather to the accumulation of carbohydrates microbial cells. If degradation was very slow, consumption would be depressed, and if the

rate of degradation slowed, then the amount of nutrients could avoid fermentation in the rumen. Based on the calculation of the inflection point on the curve obtained, the value of VFA (25;8), showed that the optimum level of garlic husk extract, was not the level of 25 ppm. The relationship between total VFA (Y) with the level of the garlic husk extract (X) was a cubic shaped. The relationship was formulated as $Y = 203.16 - 3.2646X + 0.2447 X^2 - 0.0033X^3$ (with a coefficient of determination, $R^2 = 98.77\%$)

Protozoa Count

The results of analysis of variance showed that the treatment effect was very significant ($P < 0.01$) on the number of protozoa. The highest average value obtained in the treatment of R0, which amounted to 3.80 ± 0.32 (10^6 cells/ml rumen fluid) and the average number of protozoa in treatment R4, which amounted to 2.35 ± 0.37 (10^6 cells/ml rumen fluid). Protozoa population decreased due to the reduce population of methanogens, that there is no difference between the control diet R0 and R1 (R0 + Cr 1.5 ppm + 40 ppm Zn lysinat + 0.3 ppm Se). But there was a difference in the decrease in protozoa (R0 vs. R2, R3, R4, R5) (3.80 ± 0.32 vs 2.66 ± 0.34 ; 2.52 ± 0.13 ; 2.35 ± 0.37 ; 2.71 ± 0.72). Feed R1 is not supplemented with a garlic husk extract, whereas R2, R3, R4, R5 is equipped with extract of garlic husk contains allicin and organosulfur at different levels. Protozoa population declined due to the increased of allicin and organosulfur as the anti-methanogenic, but supplemented up to 45 ppm of garlic husk extract not effective.

The addition of garlic husk extract and organic minerals in this study (Table 2) were able to reduce the population of protozoa. Test Results Honestly Significant Difference (HSD) showed that there was no difference between the control diet R0 and R1 treatment (R0 + Cr + 1.5 ppm 40 ppm Zn lysinat + 0.3 ppm Se). But there is a difference in the decrease in protozoa

Table 2. Effect of treatment on protozoa population

Treatments	Protozoa population (10^6 cells/ml rumen fluid)	Decreased (%)
R0	3.80 ± 0.32^e	-
R1	2.92 ± 0.41^{abcde}	23.20
R2	2.66 ± 0.34^{abc}	30.10
R3	2.52 ± 0.13^{ab}	33.72
R4	2.35 ± 0.37^a	38.16
R5	2.71 ± 0.72^{abcd}	28.78

Values bearing different superscript at the same column shows significant ($P < 0.05$).

R0: control feed, R1: R0 + Cr + 1.5 ppm 40 ppm Zn lysinate + 0.3 ppm Se, R2: R1 + 15 ppm garlic husk extract (*Allium sativum*), R3: R1 + 30 ppm garlic husk extract, R4: R1 + 45 ppm of garlic husk extract, R5: R1 + 60 ppm of garlic husk extract.

(R0 vs. R2, R3, R4, R5) (3.80 ± 0.32 vs 2.66 ± 0.34 ; 2.52 ± 0.13 ; 2.35 ± 0.37 ; 2.71 ± 0.72). R1 feed is without a garlic husk extract, while the feed of R2, R3, R4, R5 are supplemented garlic husk extract with allicin and organosulfur containing with different levels.

The average value of R5 (R1 + 60 ppm garlic husk extract) provided enhanced protozoa in population although similar to R0 and R1 ($R5 > R2, R3, \text{ and } R4$). This was presumably because at the level of 60 ppm active ingredient (allicin) did not only suppress methanogens, but also other bacteria. Yokohama et al. (1994) stated that the protozoa and bacteria competed in using dietary energy, in which the former would use bacteria as a source of protein for life so that the organic mineral supplementation and the garlic husk extract at 0 ppm (R1), 15 ppm (R2), 30 ppm (R3), 45 ppm (R4), and 60 ppm (R5) in feed of dairy goats was able to reduce the population of protozoa as much as 23.2, 30.1, 33.72, 38.16 and 28.78%, respectively. The decrease was assumed to be organosulfur and allicin content in the garlic husk extract as antimetagen to inhibit the growth of protozoa. Ankri and Mirelman (1999) stated that the allicin content in the form of pure compounds will be antibacterial activity against gram positive and gram negative and as an antiparasitic against protozoa parasites.

Orthogonal polynomials test results showed that the population of protozoa in all treatments were not significantly different ($P > 0.05$), because there was no significant

difference in all responses (R1-5 Linear, quadratic R1-5, R1-5 Cubik, and R1- 5 Quartik). This shows that all levels of garlic husk extract supplementation is able to reduce the population of protozoa.

Total Gas

The results of analysis of variance showed that significant effect ($P < 0.05$) in total gas production, which means that the garlic husk extract supplementation and micro minerals affect the total gas production in dairy goats. The highest average value of total gas production was achieved by R0, 27.71 ± 3.68 ml/g of feed and the lowest average value of the total gas production was achieved by R3, 21.06 ± 2.04 ml/g of feed.

Table 3 shows that the addition of garlic husk extract and organic minerals in this study were able to lower the total gas production. The mean value of the total gas treatment decreased from 27.71 ± 3.68 ml/g of feed in control (R0) to 21.06 ± 2.04 in feed of R3 (R1 + 30 ppm of garlic husk extract). The use of garlic husk extracts with the highest level of 60 ppm (R5) resulted in lower total gas compared to control diet (R0), but it produced higher total gas than R1, R2, R3, and R4. It was caused by the level of excess garlic husk extract increase in other gases and thereby less efficient in total gas reductions. Therefore, although methane can be suppressed, other gases such as CO_2 , N_2 , and O_2 in the rumen could increase as a result of R5 treatment (R1 + 60 ppm of garlic husk

Table 3. Effect of supplementation of garlic husk extract and organic mineral on total gas production

Treatments	Total Gas	Decreased (%)
R0	27.71±3.68 ^C	-
R1	24.28±2.13 ^{abc}	12.4
R2	22.48±1.21 ^{abc}	18.85
R3	21.06±2.04 ^a	24
R4	21.63±5.58 ^{ab}	21.92
R5	24.34±6.0 ^{abc}	12.14

Values bearing different superscript at the same column shows significant ($P < 0.05$).

R0: control feed, R1: R0 + Cr + 1.5 ppm 40 ppm Zn lysinate + 0.3 ppm Se, R2: R1 + 15 ppm garlic husk extract (*Allium sativum*), R3: R1 + 30 ppm garlic husk extract, R4: R1 + 45 ppm of garlic husk extract, R5: R1 + 60 ppm of garlic husk extract.

Extract). Arora (1989) stated that the gas formed from the rumen fermentation process consisted of 56% CO₂, 32% methane (CH₄), 8.2% O₂ and 3.5% N₂.

Organic micro mineral supplementation and the garlic husk extract 0 ppm (R1), 15 ppm (R2), 30 ppm (R3), 45 ppm (R4), and 60 ppm (R5) in feed of dairy goats was able to lower the total gas each as much as 12.4%; 18.85%; 24%; 21.92% and 12.14%, respectively. Machmuller et al. (2003) stated that the methanogens produced from ectosymbiosis relationship with protozoa in the interspecies transfer of hydrogen was between 9 to 25%. This was supported by Anassori et al. (2012) that H₂ and CO₂ from the fermentation of feed in the rumen was converted to CH₄ by methanogenic through interspecies hydrogen transfer, thus decreasing the number of protozoa will cause a decrease in methane production.

Based on the calculation of the inflection point on the curve, obtained value of the total gas (30.66;21.19) showed that the optimum level of garlic husk extract was 30.66 ppm level or at the level of 31 ppm. The relationship between the total gas production (Y) with garlic husk extract (X) could be formulated with the equation $Y = 24.476 - 0.2146X + 0.0035X^2$ (coefficient of determination, R^2 : 0.96%). Evidently, declining protozoa population was due to the active substance and organosulphur allicin from garlic husk extract that could inhibit the enzyme HMG-CoA performance in

suppressing the methanogenic population; therefore, the population of protozoa and total gas production decreased. De Rosa et al. (1986) stated that the methanogens have lipid membranes composed of glycerol or complex bond with the formation of isoprenoid alcohols catalyzed by the enzyme 3-hydroxy-3methyl-glutaryl coenzim A (HMG-CoA). Busquet et al. (2006) reported that organosulfur was capable of inhibiting the enzyme HMG-CoA therefore methanogens lipid membrane was not formed.

Conclusion

The research showed that supplementation of garlic husk extract in the diet of dairy goats as much as 25 ppm decrease DMD, OMD, the total gas, and protozoa as well as increased the concentration of total VFA. Conclusively, 25 ppm was the optimum level of supplementation with garlic husk extract (*Allium sativum*) to improve the efficiency of rumen fermentation of dairy goat.

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