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Research Article
Effect of Garlic Extract and Organic Mineral Supplementation on Feed Intake, Digestibility and Milk Yield of Lactating Dairy Cows

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Abstract

Background and Objective: The objective of this study was to evaluate supplementation of garlic (*Allium sativum*) extract and organic minerals (1.5 ppm organic Cr, 0.3 ppm organic Se and 40 ppm Zn-lysinat) on milk yield, milk quality and feed digestibility of lactating dairy cows. **Materials and Methods:** Fifteen multiparous, one month pre-partum Friesian Holstein cows with an average body weigth of 638±72 kg were used as the experimental units and therefore, there were 5 replicates for each treatment. The cows were fed with a Total Mixed Ration (TMR) consisted of Napier grass: Concentrate mixture (60:40 w/w, dry matter basis) as the basal diet. The experiment was conducted with three treatments, namely control feed (CTL), control feed+Organic Minerals (OM) and OM+garlic extract (OM-G). Variables measured were nutrient intake, nutrient digestibility, milk yield and milk composition. Data were analyzed by analysis of variance with a completely randomized design. **Results:** Supplementation of garlic extract significantly influenced the digestibility of Crude Fiber (CF), Neutral Detergent Fiber (NDF), milk yield and milk efficiency ($p<0.05$). However, the supplementation had no effect on nutrient intake (dry matter, organic matter and total digestible nutrient) and milk composition (fat, protein, lactose and solid non-fat). Supplementation of garlic extract and organic mineral resulted in better feed digestibility, milk production and milk efficiency than those of the control diet and control diet supplemented with organic mineral ($p<0.05$). **Conclusion:** Supplementation of combined garlic extract and organic mineral (Se, Cr and Zn) in dairy cows increases feed digestibility, milk production and milk efficiency.

Key words: Herbal, Se-Cr-Zn organic, milk efficiency, crude fiber, *Allium sativum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, a lot of research has been focused on evaluating the potential of plant extracts as alternative to antibiotics in order to improve feed efficiency in ruminants. Plants produce various secondary metabolites that might have antimicrobial activities against certain groups of rumen microbes (Benchaar *et al*., 2008; Jayanegara *et al*., 2012, 2014; Prayitno *et al*., 2015). Garlic (*Allium sativum*) is a plant herb that contains high levels of bioactive substances particularly organosulphur compounds, such as allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan (Lawson, 1998). Garlic has been widely used as an antibacterial agent and has been extensively used to maintain the microbial ecosystem of the gastrointestinal tract especially in tropical regions. Several studies had shown positive impacts of garlic addition on ruminants such as by decreasing acetate to propionate ratio in the rumen (Busquet *et al*., 2006), improving the efficiency of rumen fermentation, reducing enteric methane emissions (Prayitno *et al*., 2013; Wanapat *et al*., 2013), improving the performance of dairy cows (Yang *et al*., 2007), buffaloes, beef cattle (Kongmun *et al*., 2010, 2011) and dairy goats (Prayitno *et al*., 2014).

On the other hand, in tropical countries such as Indonesia, mineral supplementation is essential due to the typically low concentration of mineral in forages, including trace elements (Little, 1986). Supplementation of trace elements is possible but the low bioavailability may limit their usages. A strategy to improve the bioavailability of essential trace elements is through combination with organic molecules or being incorporated into a certain microbial biomass prior to supplementation. The consideration of using organic minerals is based on the utilization and their toxicity as compared to inorganic minerals. Based on the above description, it would be interesting to investigate simultaneous addition of garlic extract and organic mineral. Therefore, the purpose of this study was to evaluate the effect of supplementation of garlic extract and organic mineral (Se, Cr and Zn) on DM, OM, NDF and TDN intake and digestibility, milk production and milk composition of lactating dairy cows.

MATERIALS AND METHODS

Preparation of experimental materials: Preparation of garlic extract was following the method described by Prayitno and Hidayat (2013). Garlic was obtained and purchased from a local market in Purwokerto, central Java, Indonesia. At the first phase, garlic was separated from the chaff and then transversally sliced with a diameter of 1 mm and dried. Dried garlic was subsequently milled. Garlic powder was then macerated for 24 h with methanol (1:4 w/v). Subsequently, the methanol was evaporated in a rotavapor and eventually the residue was separated from the solvent. The extraction procedure was repeated twice and the extract was pooled. The extract was then dried in a freeze drier and stored at -4°C until use. The organic minerals were prepared from 100 ppm of $CrCl_3$, SeO_2 and $ZnCl_2$ that were fermented using *Saccharomyces cerevisiae* for 4 days at room temperature.

Animals and dietary treatments: The study was carried out at the National Dairy centre, Baturraden, central Java, Indonesia in 2014. Fifteen multiparous, one month pre-partum Friesian Holstein cows with an average body weigth of 638±72 kg were used as the experimental units and therefore, there were 5 replicates for each treatment. The cows were fed with a Total Mixed Ration (TMR) consisted of Napier grass: Concentrate mixture (60:40 w/w, dry matter basis) as the basal diet that was formulated to contain 13.8% Crude Protein (CP), 4.2% Ether Extract (EE), 21.3% Crude Fiber (CF) and 67.0% Total Digestible Nutrient (TDN). Such formulation was based on the NRC (2001) recommendation to provide sufficient energy and protein for 650 kg cow to produce 20 kg day^{-1} of milk containing 3.5% fat and 3.2% protein. Feed and water were provided *ad libitum*. The three treatments tested were:

CTL: Basal feed
OM: CTL+organic mineral (0.3 ppm Se+1.5 ppm Cr+40 ppm Zn-lysinate)
OM-G: OM+0.25 ppm garlic extract (1.7% allicin)

The experiment was performed for 12 weeks, i.e., 4 weeks pre-partum and 8 weeks post-partum period. Adaptation period lasted for 4 weeks and followed by 7 weeks of sampling and data collection period, in which the cows were milked without colostrum collection. The cows were housed individually to prevent cross-feeding and fed the TMR twice a day at 06.00 and 12.30 h. Daily milking was done at 04.30 and 15.00 h. Cows were weighed at the beginning and 7 days after parturition. All cows were cared for in accordance with the guidelines established by National Dairy centre, Baturraden, Indonesia.

Sampling and laboratory analysis: Feed samples of TMR and ingredients were collected weekly, where orts were collected daily and composited weekly for Dry Matter (DM) determination. Feed intake was calculated as the difference between feed offered and orts. Samples of feeds and orts were composited by period, oven-dried at 55°C for 48 h and ground through a 1 mm diameter screen (Wiley mill, standard

model 4, Arthur H. Thomas Co., Philadelphia, USA) for subsequent determination of ash, CF, NDF and CP. The analysis was conducted according to the method of AOAC (2000).

Milk analysis: Milk production was recorded daily. During the last 7 weeks of the experimental period, milk samples were collected, preserved with potasium dichromate and subsequently analyzed for fat, protein and lactose contents by using a lactoscan milk analyzer. Milk samples for Somatic Cell Count (SCC) determination were collected at the 8th week post-partum and the analysis was done according to the method of Hirst *et al*. (1984).

Statistical analysis: Allocation of treatments into experimental units followed a completely randomized design since the experimental units were relatively homogenous. The data obtained were analyzed by analysis of variance (ANOVA). When the treatment effects showed significantly different at $p<0.05$, a *post hoc* test namely Duncan's multiple range test was employed. The statistical analysis was performed using SPSS statistical software version 20.

RESULTS AND DISCUSSION

Effect of garlic extract and organic mineral on feed intake and digestibility: The results did not show any significant differences among the treatments with regard to Dry Matter Intake (DMI), Organic Matter Intake (OMI) and Total Digestible Nutrient (TDN) intake (Table 1). These results indicated that the garlic extract and organic mineral supplementation did not disturb microbial activity in the rumen. Similar to the present result, Wanapat *et al*. (2013) reported that herb supplementation had no effect on DMI and nutrient digestibility except for CP digestibility. This was also in agreement with Yang *et al*. (2007) and Oh *et al*. (2013) who found that no change in DMI was observed when dairy cows were fed with the mixture of essential oil compound or garlic oil. Prayitno *et al*. (2014) found the different values of supplementation of garlic mineral mixture in dairy goat diet. In ruminant animals including dairy cows, feed intake could be influenced by a number of factors, such as body weight, lactation stage, physical fill, digestion, passage rate, or fermentation metabolites (Allen, 2000; Riaz *et al*., 2014).

Supplementation of garlic extract decreased CF and NDF digestibility, but increased TDN digestibility (Table 2). These results showed that the garlic extract supplementation tended to reduce cellulolytic bacteria activity, whereas organic mineral supplementation tended to increase cellulolytic bacteria activity. These data showed that Se, Cr and Zn were very important for cellulolytic rumen microbes. On the other hand, there was an indication about the occurence of rumen microbial activity in terms of amylolytic, proteolytic and lipolytic as indicated by the increased TDN digestibility. Yang *et al*. (2007) reported that the use of garlic as much as 5 g day^{-1} in cattle feed increased digestibility of dry matter and organic matter as compared to the control diet.

Table 1: Effect of garlic extract and organic mineral supplementation in diet on nutrient intake of lactating dairy cows

Item	CTL	OM	OM-G	p-value
Intake (kg day^{-1})				
DM	19.0±4.09	23.2±1.53	21.3±1.14	0.065
OM	14.9±3.58	18.2±1.69	16.3±0.85	0.068
NDF	5.03±0.31	5.16±0.29	5.37±0.21	0.186
TDN	41.6±5.56^{a}	50.9±2.94^{b}	43.6±3.35^{a}	0.006

CTL, Basal feed, OM: CTL+organic minerals (0.3 ppm Se, 1.5 ppm Cr, 40 ppm Zn-lysinate), OM G: OM+0.25 ppm garlic extract, DM: Dry matter, NDF: Neutral detergent fiber, OM: Organic matter, TDN: Total digestible nutrient, Means in the same row with different superscripts differ significantly ($p<0.05$)

Table 2: Effect of garlic extract and organic mineral supplementation in diet on nutrient digestibility of lactating dairy cows

Item	CTL	OM	OM-G	p-value
Digestibily of intake (%)				
DM	61.6±3.15	64.4±2.85	64.0±1.74	0.225
OM	53.6±2.40	55.5±0.79	55.3±1.17	0.141
NDF	54.9±4.14^{a}	56.0±3.7^{b}	52.1±4.42^{a}	0.005
TDN	74.1±3.58^{a}	79.8±0.85^{b}	75.7±0.69^{a}	0.003

CTL: Basal feed, OM: CTL+organic minerals (0.3 ppm Se, 1.5 ppm Cr, 40 ppm Zn-lysinate), OM G: OM+0.25 ppm garlic extract, Means in the same row with different superscripts differ significantly ($p<0.05$)

Effect of garlic extract and organic mineral on milk yield and milk composition: Supplementation of garlic extract and organic mineral significantly increased milk production ($p<0.05$, Table 3). This indicated that supplementation of garlic extract in feed containing adequate Cr, Se and Zn-lysinate was able to increase milk synthesis. Selenium in the form of physiological GSH-Px protects the secretory cells of mammary gland from the free radical that are produced during metabolism, therefore the integrity of secretory cells is warranted. The addition of minerals, such as Cr increases insulin receptors of secretory cells in mammary gland, while Se improves udder gland secretory cells. The increase of insulin receptors of secretory gland cells will bind greater concentration of glucose. Because glucose is the precursor of lactose synthesis and has ability to bind water, thus milk production increases (Prayitno *et al*., 2014). Smith *et al*. (2008) reported that Cr methionine supplementation (CrMet) increased milk production. This is due to that the udder secretory cells do not have insulin receptors and in the

Table 3: Fffect of garlic extract and organic mineral supplementation in diet on daily milk yield and milk component of lactating dairy cows

Item	CTL	OM	OM-G	p-value
Actual milk yield (kg)	13.8±2.48[a]	15.9±2.23[a]	19.2±0.49[b]	0.003
Milk yield (kg 4% FCM)	12.9±2.04[a]	14.9±1.42[a]	20.1±1.23[b]	<0.001
Milk composition (%)				
Fat	3.55±0.21	3.53±0.24	3.71±0.24	0.051
Protein	2.78±0.18	2.96±0.08	2.85±0.07	0.094
Lactose	4.05±0.15	4.18±0.20	4.12±0.15	0.445
Solid Non Fat (SNF)	7.94±0.12	8.01±0.23	8.06±0.19	0.371
Production (g day^{-1})				
Fat	491±97.5[a]	564±94.4[a]	714±10.5[b]	0.002
Protein	439±77.8[a]	570±22.8[b]	548±29.9[b]	0.003
Lactose	561±116[a]	664±112[b]	793±29.2[b]	0.003
SNF	1095±205[a]	1278±203[a]	1551±67.6[b]	0.004
SCC (10^5 cell mL^{-1})	3.48±0.18[b]	2.08± 0.18[a]	2.05± 0.13[a]	<0.001
Glucose blood (mg dL^{-1})	73.3±2.52[b]	54.0±1.04[a]	48.7±1.53[a]	0.003

FCM: Milk×(fat%×0.15+0.4), Means in the same row with different superscript differ significantly (p<0.05)

presence of Cr, the insulin sensitivity increases and subsequently the milk production increases (Prayitno *et al*., 2014; Hayirli *et al*., 2001).

Supplementations of garlic extract and organic minerals were able to guarantee the optimum activity of milk production as it was shown by the decrease in the number of somatic cells in the milk as much as 40.57%. The milk somatic cell count is a good indicator to determine the health status of the udder, especially of subclinical mastitis. The selenium supplementation irrespective of source, tends to reduce the prevalence of intramammary infection (IMI) and to decrease the prevalence of quarters to high Somatic Cell Count (SCC) at calving (Weiss and Hogan, 2005; Ceballos-Marquez *et al*., 2010) and selenomethionine increases proliferation and reduces apoptosis in bovine mammary epithelial cells under oxidative stress (Miranda *et al*., 2011). These data informed, although there was an increase in milk production, however, half-life of secretory cells of mammary glands was constantly kept in term of its integrity. The increase of milk production was possible because garlic has anti-methanogenic characteristic (Kongmun *et al*., 2010; Hart *et al*., 2006) and therefore, the energy of approximately 130 KJ mol^{-1} CH_4 that usually used for methane synthesis during ruminal fermentation (Sahakian *et al*., 2010) can be converted for milk production. This case shows that garlic extract increases rumen fermentation efficiency. On the other hand, the organic mineral supplementation was able to increase organic matter digestibility, therefore the availability of substrates for milk synthesis was in optimum condition. Yang *et al*. (2007) showed that garlic supplementation as much as 5 mg/cow/day in dairy cattle showed a little increase of milk production relative to that of control (29 vs 29.9 kg day^{-1}). This case showed that garlic supplementation was only able to save digestible energy as much as 10% that usually lose during methane synthesis. In tropical countries like Indonesia, when almost all of the available feedstuffs are deficient in minerals, mineral supplementation in the form of organic that is combined with garlic extract is able to increase the efficiency of energy and milk production.

Supplementation of garlic extract plus organic minerals increased the production of fat, protein, lactose and SNF, although the percentages of milk components were similar among the three treatment (Table 3). The supplementation of garlic extract plus organic mineral was able to increase the four component of milk more than 60%.

Effect of garlic and organic minerals on milk efficiency: The results showed that supplementation of garlic extract and organic minerals (Se, Cr and Zn) increased milk efficiency, energy efficiency and decreased the ratio of DMI: Milk production (Table 4). Efficiency is related to how much milk production quantity is able to be produced from the total feed intake (Prayitno *et al*., 2014). The supplementations of combined garlic extract and organic mineral increase significantly milk efficiency by 17.43% relative to control fed. Similar result was obtained, namely the ratio of DMI: Milk yield decreased by supplementation of garlic extract and organic mineral in the fed. Prayitno *et al*. (2014) reported that supplementation of garlic powder and organic mineral in dairy goat increased milk efficiency by as much as 48%.

The blood glucose reduction 3 h of post-feeding, showed that the glucose uptake by target cells (including secretory cells of the mammary gland) increased. The glucose reduction was followed by the increase of milk lactose. This shows that the glucose that enters the secretory cells of the mammary gland can be synthesized into lactose by the availability of galactose in the blood. These data show that the organic Cr supplementation in diet containing garlic extract has a major role in the activation of insulin, thus it increases glucose

Table 4: Effect of garlic extract and organic mineral supplementation on milk efficiency and energy efficiency

Item	CTL	MO	MO-G	Prob.
Ratio of DMI: Milk yield	1.37±0.11[b]	1.41±0.14[b]	1.11±0.08[a]	0.001
Milk efficiency (%)	73.6±6.87[b]	68.4±6.39[a]	90.1±6.22[c]	<0.001

Means in the same row with different superscript differ significantly (p<0.05)

uptake. The increase of synthesis efficiency of milk suggests that a combination of organic minerals (Se, Cr and Zn) with extracts of garlic in the dairy cows diet has a major role in optimizing the milk production.

CONCLUSION

The supplementation of combined garlic extract and organic mineral (Se, Cr and Zn) in dairy cows increases feed digestibility, milk production and milk efficiency.

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SIGNIFICANCE STATEMENTS

- Use of antibiotics as additives to improve animal productivity have been banned in many countries
- Garlic extract is potential to be used as an alternative to antibiotics in improving feed efficiency and production performance of lactating dairy cows
- Low concentrations of minerals in tropical forages have to be surmounted by supplementation
- Supplementation of minerals bound with organic materials improves their availability in the digestive tract of animals
- Combination between garlic extract and organic minerals synergistically improved milk yield, feed efficiency and reduced milk somatic cell count of lactating dairy cows

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