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## Research Article

# Effects of Volatile Oil of Garlic on Feed Utilization, Blood Biochemistry and Performance of Heat-stressed Japanese Quail

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## Abstract

**Background and Objective:** The heat stress of quail plays an important role in feed utilization, blood profile, growth performance and its influence economic loss. Supplemented of volatile oil garlic is one the efforts to avoid the negative impact of heat stress. This study was conducted to investigate the potential of garlic powder in improving feed utilization, some parameters of blood biochemistry and performance. **Materials and Methods:** A total of 400 laying quails was used in this experiment. Animal samples were obtained at 6 week of age randomly assigned to 4 groups of treatment. Each group of treatment involved 10 replicates with 10 quails each (100 laying quails per group). All of the groups were provided basal diet and supplemented with volatile oil of garlic 0 (control), 0.75, 1.50 and 2.25 mL L<sup>-1</sup> drinking water for group of D0, D1, D2 and D3, respectively. All of the groups were exposed heat stress under 33-35°C. Faces and eggs were collected and also measured feed consumption to determined feed utilization. Blood sample was collected and centrifuged to separate plasma. The plasma was used for determination of concentration of blood biochemistry by spectrophotometer method, based on BIOLABO kit. Data were analyzed by one-way ANOVA using SAS. **Results:** Based on the results of this study, there was significant effect ( $p < 0.05$ ) supplementation of volatile oil garlic on the feed utilization between D0 and D1, D2, D3. Commonly, supplemented of volatile oil garlic showed significantly affected ( $p < 0.05$ ) on the blood biochemical and performance, however did not significantly affected ( $p > 0.05$ ) on the glucose and low density lipoprotein (LDL). The administration of 2.25 mL L<sup>-1</sup> drinking water was a optimum level for increasing feed utilization, improving profile of blood biochemistry and rising performance. **Conclusion:** The result in this study indicated that bioactive compound in volatile oil can be improved feed utilization, metabolism rate and quail production by administration 2.25 mL L<sup>-1</sup> in drinking water.

**Key words:** Quail, bioactive compounds, heat-stressed, metabolism, basal diet

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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

Quail is a highly nutritive and rich source of animal protein. Quail is one breed poultry which is reared in Indonesia, but this animal needs the neutral ambient temperature is called thermoneutral zone. Temperature play the main role on physiology of poultry, previously study reported damage a thermoregulation in hen and quail stressed high temperature. The appropriate range of the housing temperature for poultry is 20-25°C<sup>1,2</sup>. This thermoneutral zone is temperature range for maintenance of normal metabolism rate or physiological profile of poultry, included quails.

The lower or higher temperature not only showed a decrease in physiological status of animal but also decline the performance<sup>3</sup>, immunity<sup>4,5</sup> and feed utilization<sup>6-8</sup>. Many investigators showed decline of economic value and health status in their previous study on the Indonesian breed poultry as a consequence of high temperature.

On the other hand, the main aspect which plays role in poultry production is feed management. A good feed management not only avoided heat stress but also be able to improve poultry performance. Many researchers have reported various activities on natural substances like anti-stress, to enhance performance and growth promotion of animal stressed with temperature. Studies demonstrated that aromatic plant like garlic was an effective alternative to synthetic antibiotic in encouraging layer performance<sup>9-11</sup> and broiler<sup>12,13</sup>.

Many reports have concluded that garlic showed significant effect in various poultry species such as laying hen, broiler. But only limited published and information is available about supplementation of volatile oil of garlic (VOG) for laying quail. Therefore, the current experiment was evaluated the potential of VOG in improving feed utilization, some parameter of blood biochemistry and performance.

## MATERIALS AND METHODS

This study was carried out in Animal Station, Department of Animal Nutrition and Feed Technology, Animal Science Faculty, Universitas Padjadjaran (Unpad), Campus of Jatinangor, Sumedang West Java and Farm Animal of Quail Breeding, Cikole, Sukabumi, during period from January-July, 2017.

**Birds, experimental design and treatments:** This study was carried out at the Laboratory of Animal Physiology and

Biochemistry and the Poultry Housing, Animal Station of Animal Science Faculty, Universitas Padjadjaran. In this experiment, 400 laying quail (*Coturnix coturnix*) were obtained at 6 weeks of age were randomly assigned to 4 groups of treatment, with 33-35°C housing temperature. Each group of treatment involved 10 replicates with 10 quails each (100 laying quails per group).

A diet composition as basal ration, consist of corn-soybean, fish meal, bone meal, rice polish, coconut oil, with nutrient composition was ME 2800 kcal kg<sup>-1</sup>, CP 18.4%, Ca 3.5% and available phosphorus 0.35%. Feed and water was provided *ad libitum* and diet were presented in mash form.

Fresh garlic bulbs were obtained from garlic farmer, District of Tegal, Centre of Java Indonesia. Garlic was prepared as described by Kasuga *et al.*<sup>14</sup>. In isolation of volatile oil of garlic bulb using water distillation, that was sample contact with water directly. The heating, the oil will evaporate along with the water through the condenser, the oil mixed with water collected in erlenmeyer flask. The result was separated by using separating funnel and inserted into the vial, added anhydrous Na<sub>2</sub>SO<sub>4</sub> to remove the water contained in the volatile oil of garlic (VOG). Composition of volatile oil was determined using gas chromatography mass spectrometer (GC-MS). The volatile oil content of garlic was shown in Table 1.

All of the group was provided basal diet and supplemented with 0 (control), 0.75, 1.50 and 2.25 mL VOG L<sup>-1</sup> drinking water, for group of D0, D1, D2 and D3, respectively.

**Sample collection and analytical determination:** Feces was collected based on method from Abun<sup>15</sup> with using a lignin indicator used in this study. Feces samples were used to measure digestibility of dry matter, organic matter, protein and nitrogen retention.

Table 1: Volatile oil composition of garlic

Composition	Percentage
Diallyl sulfide	4.83
Diallyl disulfide	30.08
Diallyl trisulfide	11.84
Diallyl tetrasulfide	1.09
Methyl allyl disulfide	3.84
Methyl allyl trisulfide	3.98
Citronella	11.25
β-citronellol	7.38
Isopulegol	0.50
Geraniol	13.36
Neryl acetate	3.73
Citronellyl acetate	3.86

Blood was also collected from the wing vein of the same laying quails that were selected at random from each treatment group (three quails per replication) on week 0, 4 and 8 of the experiment, using a sterilized syringe and vacuum tube contain  $K_3$ -EDTA. The blood sample collected was centrifuged to separate the plasma. The plasma was used to determine the concentration of blood biochemistry by automatic biochemical analyzer.

Eggs were collected and labeled on daily basis at treatment group also, the percentage of egg production and egg weight were recorded daily. To know feed conversion, also feed intake was measured.

**Statistical analysis:** The data were statistically analyzed by one-way analysis of variance (ANOVA) using the GLM procedure of SAS Version 8.2<sup>16</sup> for a completely randomized design. The VOG levels in the drinking water were used as class statement. The results of feed utilization, blood parameter and performance were subjected to analysis of variance. Difference among the means of all treatments was separated by Duncan's multiple range tests (SAS Institute) at a 95% significant level.

## RESULTS AND DISCUSSION

**Feed utilization:** Dry matter digestibility and protein digestibility not significantly influenced by administration with VOG in drinking water (Table 2) in this experiment. Significant differences in organic matter digestibility, nitrogen retention and feed conversion were found, however, there is no statistical difference among VOG groups. Similarly affect based on in this study have reported Alcicek *et al.*<sup>6</sup> in broiler, Mahmoud *et al.*<sup>7</sup> in layer hens and also Lee *et al.*<sup>17</sup> in juvenile sterlet sturgeon.

Enhancing feed utilization is a major factor in reducing the cost of quail production and poultry production generally<sup>18</sup>. In the current study, a positive effect by using VOG on the feed utilization in quails was observed. Garlic has been used for human and animal. Previous study had reported that garlic products showed a broad antibiotic spectrum against both gram-positive and also gram-negative bacteria<sup>19-23</sup>. In the

same way this study results, also showed by Olobatoke and Mulugeta<sup>11</sup>, dietary garlic contributed to decline intestinal bacteria.

An increase in feed utilization or efficiency of nutrient absorption, associated with lower numbers of intestinal pathogens microorganism<sup>24-27</sup>. In current investigation, the rise feed utilization similar with the other studies reported previously. Lee *et al.*<sup>17</sup> showed a rise of feed digestibility by dietary garlic in sturgeon and high feed efficiency in laying hens<sup>26</sup> and broiler<sup>28,29</sup>.

In addition, an increase feed utilization based on the result of this research, also associated with increased absorptive area of the intestine. Peinado *et al.*<sup>22</sup> reported that supplementation of garlic derivative propyl propane thiosulfonate (PTS) created a rise to the surface area of the villi. Van Leeuwen *et al.*<sup>30</sup> showed PTS play a role for decreasing intestinal pathogen microbes and lead to growth intestine. The other investigator, Adibmoradi *et al.*<sup>31</sup> also showed that garlic supplementation enhanced villus height and crypt depth and decline epithelial thickness and increase goblet cell numbers in duodenum, jejunum and ileum of bird, similar results, have reported by Horn *et al.*<sup>32</sup> and Nusairat<sup>33</sup>. It was known that a progressive increase both in absorptive area and in the mucosal capacity for hydrolysis. These phenomena gives rise to gain nutrient absorption and feed utilization generally.

**Blood parameter:** Data in Table 3 showed the effect of garlic powder supplementation with different levels on blood parameter of laying quail. The supplementation with 0, 0.75, 1.50 and 2.25 mL, VOG had significantly affected ( $p < 0.05$ ) on blood parameter. Concentration cholesterol and triglyceride were significantly affected by supplementation of VOG levels, whereas, there was no significant difference in glucose content among the quail samples ( $p > 0.05$ ).

The glucose concentration presented no significant in this current study supported with previous study<sup>17</sup>, which reported that glucose play the main role to maintain blood pressure and osmolarity. The homeostasis role of insulin, facilitates glucose uptake and incorporation into glycogen. Insulin also regulates plasma concentration of glucose<sup>34</sup>.

Table 2: Effect of VOG levels on feed utilization of laying quail

Indices	Diet			
	D0	D1	D2	D3
Dry matter digestibility (%)	36.97 ± 1.05 <sup>a</sup>	37.84 ± 2.84 <sup>a</sup>	36.67 ± 2.73 <sup>a</sup>	36.45 ± 1.82 <sup>a</sup>
Protein digestibility (%)	42.16 ± 1.84 <sup>a</sup>	43.98 ± 2.52 <sup>b</sup>	42.58 ± 1.39 <sup>b</sup>	43.39 ± 1.46 <sup>b</sup>
Organic matter digestibility (%)	40.54 ± 0.98 <sup>a</sup>	44.83 ± 0.68 <sup>b</sup>	43.53 ± 0.46 <sup>b</sup>	43.98 ± 0.68 <sup>b</sup>
Nitrogen retention (%)	38.04 ± 1.14 <sup>a</sup>	40.93 ± 0.89 <sup>b</sup>	39.78 ± 0.78 <sup>b</sup>	39.74 ± 1.03 <sup>b</sup>
Feed conversion, g of feed/g of egg	3.46 ± 0.37 <sup>a</sup>	2.89 ± 0.92 <sup>b</sup>	2.98 ± 0.96 <sup>b</sup>	2.97 ± 0.45 <sup>b</sup>

<sup>a,b</sup>Means in each row with different superscripts are significantly different ( $p < 0.05$ ). Value are given in Means ± SD

Table 3: Effect of VOG levels on some biochemical parameters of laying quail blood plasma

Indices	Diet			
	D0	D1	D2	D3
Glucose (mg dL <sup>-1</sup> )	289.85±5.38 <sup>a</sup>	290.62±4.51 <sup>a</sup>	289.04±6.46 <sup>a</sup>	290.39±5.19 <sup>a</sup>
Cholesterol (mg dL <sup>-1</sup> )	257.56±1.47 <sup>a</sup>	123.67±1.82 <sup>b</sup>	124.48±2.02 <sup>b</sup>	124.73±1.38 <sup>b</sup>
Triglycerides	33.43±1.67 <sup>a</sup>	32.98±1.58 <sup>a</sup>	33.14±1.04 <sup>a</sup>	31.07±0.92 <sup>b</sup>
LDL (mg dL <sup>-1</sup> )	58.26±4.03 <sup>a</sup>	56.63±5.07 <sup>a</sup>	49.75±4.01 <sup>a</sup>	54.63±3.07 <sup>a</sup>
HDL (mg dL <sup>-1</sup> )	76.91±1.26 <sup>a</sup>	85.93±1.05 <sup>b</sup>	122.84±1.92 <sup>c</sup>	127.93±1.02 <sup>d</sup>
Creatinine (mg dL <sup>-1</sup> )	5.82±0.15 <sup>a</sup>	5.52±0.09 <sup>a</sup>	4.13±0.17 <sup>b</sup>	4.03±0.13 <sup>c</sup>
Malondialdehyde (MDA) (μmol mL <sup>-1</sup> )	2.22±0.27 <sup>a</sup>	2.04±0.14 <sup>b</sup>	1.94±0.18 <sup>b</sup>	1.47±0.51 <sup>c</sup>
Lactic dehydrogenase (mg dL <sup>-1</sup> )	0.31±0.02 <sup>a</sup>	0.24±0.01 <sup>b</sup>	0.21±0.01 <sup>c</sup>	0.25±0.02 <sup>b</sup>
Albumin (mg dL <sup>-1</sup> )	3.83±0.13 <sup>a</sup>	3.89±0.24 <sup>b</sup>	4.28±0.14 <sup>c</sup>	4.72±0.17 <sup>d</sup>
Globulin (mg dL <sup>-1</sup> )	0.31±0.02 <sup>a</sup>	0.84±0.05 <sup>b</sup>	1.82±0.11 <sup>b</sup>	1.85±0.08 <sup>b</sup>
Total protein (mg dL <sup>-1</sup> )	4.14±0.11 <sup>a</sup>	4.73±0.08 <sup>b</sup>	6.10±0.09 <sup>c</sup>	6.58±0.10 <sup>c</sup>

<sup>a,b,c</sup>Means in each row with different superscripts are significantly different ( $p < 0.05$ ). Value are given in Means  $\pm$  SD

In this experiment, the supplementation with 0.75 g (D1) to 2.25 g VOG (D3) resulted in significantly lower cholesterol and triglyceride levels compared with the control (D0). This could be explained by the decrease of synthetic enzyme activity, because VOG would be expected to have a much higher antioxidant capacity<sup>31,35</sup> and biological effects<sup>36</sup>. Based on the result of this study garlic has potential hypocholesterolemic and hypolipidemic<sup>37,38</sup>. These possibilities was the result that there are two components of garlic, S-allyl cysteine sulfoxide (allicin)<sup>29,34</sup>. Studies as described previously indicated that allicin and alliin were potentially active compound of garlic, has promise to inhibit lipid synthesis enzymes.

The decline of plasma cholesterol and triglyceride concentration by VOG supplementation in drinking water might be due to the reduction of synthetic enzymes. The effect garlic compound inhibition of the hepatic  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7 $\alpha$ -hydroxylase and fatty acid synthase were reported<sup>37,38</sup>. Ao *et al.*<sup>34</sup> reported that hepatic  $\beta$ -hydroxy- $\beta$ -methylglutaryl co-enzyme A (HMG-CoA) reductase, cholesterol 7 $\alpha$ -hydroxylase and fatty acid synthase in pentose-phosphate pathway activities were significant decrease. Although, He *et al.*<sup>39</sup> reported a rise activities of glycerol-3-phosphate dehydrogenase for synthesis of triglyceride.

The results of this experiment did not detect significant reduction in LDL contents as a result of VOG administration contrary to what one would expect (Table 3). The lack of effect of VOG on LDL differs from the results of previously study. Chowdhury *et al.*<sup>40</sup> and Adriani *et al.*<sup>28</sup>, who reported a reduction in serum LDL linearly with increasing levels of garlic paste in laying hens for 6 weeks.

The blood creatinine of quail significantly ( $p < 0.05$ ) decreased with the addition of VOG up to 2.25 mL. Decreased creatinine with VOG showed that VOG could be decreased the hydrolysis of creatine by creatine kinase to creatinine.

Similarly, the same result in this study showed on MDA and lactic dehydrogenase level. Although, the greatest decrease in lactic levels occurred in the quail group which supplemented 1.5 mL VOG in drinking water.

The result of this experiment (Table 3) detected that the rate of anaerobic glycolysis also decreased with administration of VOG. It was showed by decreased enzyme of lactic dehydrogenase level. This enzyme plays a role to convert pyruvate into lactate without oxygen. Similarly result also showed on MDA. The MDA can be described free radical concentration in body. Based on these results, the authors speculate that also believe that this explanation might apply as antioxidant properties. Some studies have showed that garlic found significant effect in MDA and free radical<sup>31,32,41</sup>.

The potential of VOG stimulated quails immunity showed by the albumin, globulin and blood total protein significantly ( $p < 0.05$ ) increased with supplemented of VOG. Albumin, globulin and protein have been a slight increase in concentration with administered of VOG in drinking water up to 2.25 mL L<sup>-1</sup>. Several studies showed that the garlic compounds were capable of providing an increase protein related in immunoglobulin production<sup>12,17,42</sup>.

**Performance:** The effects of VOG on performance of laying quail were shown in Table 4 that the daily egg mass and egg production were significantly affected ( $p < 0.05$ ) by administration treatment. The data in Table 4 illustrates that 3 daily egg mass has raised significantly with supplemented 0.75 g VOG (D1) higher than without supplementation VOG (D0) and then fall slightly and remain the same D0. Table 4 also present egg production, there has been a slight increased in numbers with supplementation 0.75 g VOG and then showed remained stable of egg production with level of 1.50 and 2.25 mL VOG. However, the increase in hatchability of quail egg which supplemented of 1.50 mL VOG highest in among group levels.

Table 4: Effect of VOG levels on performans of laying quail

Indices	Diet			
	D0	D1	D2	D3
Egg weight (g)	10.75±0.73 <sup>a</sup>	12.15±0.92 <sup>b</sup>	11.71±1.02 <sup>b</sup>	11.03±0.52 <sup>a</sup>
Hen-day egg production (%)	75.29±1.64 <sup>a</sup>	80.16±1.93 <sup>b</sup>	79.35±2.04 <sup>b</sup>	79.49±1.84 <sup>b</sup>
Fertility (%)	84.84±1.94 <sup>a</sup>	93.46±1.28 <sup>b</sup>	93.74±1.04 <sup>b</sup>	92.74±1.03 <sup>c</sup>
Hatchability (%)	69.76±1.04 <sup>a</sup>	79.84±1.94 <sup>b</sup>	83.62±2.03 <sup>c</sup>	80.18±1.94 <sup>b</sup>

<sup>a,b,c</sup>Means in each row with different superscripts are significantly different (p<0.05). Value are given in Means±SD

These results demonstrate that supplementation VOG contributes to rise egg mass, egg production and egg hatchability. In agreement with the current study, some researchers<sup>7,37,38</sup>, presented that egg weight and egg production significantly affected by dietary garlic supplementation. However, some investigators<sup>40,43,44</sup> founded that garlic product has not effect on egg weight. The diversity of garlic preparation and supplementation method brings about contrast of result in this study with those in literature. Interestingly, some previously studies are reported that egg quality and production increased with supplemented of natural matter which contain chemical compound similar with garlic<sup>45-48</sup>.

### CONCLUSION

The result of this study showed that supplementing VOG of 2.25 mL L<sup>-1</sup> in drinking water could improve with significant impact on feed utilization, metabolism rate and also production qualities in quails.

### SIGNIFICANCE STATEMENTS

This study discovers the VOG in drinking water positively influenced in the metabolism and performance of heat-stressed Japanese quail. This study will help the researcher to uncover the critical areas of physiological quail stress of temperature that many researchers were not able to investigate. The results of this study may also contributes to the effective strategies of quail produce in tropical region.

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