



Effect of Garlic Extract (*Allium sativum*) on Hematological and Clinical Chemistry Parameters of Laying Quail

Nanik Hidayatik¹, Gandul Atik Yuliani¹, Bodhi Agustono², Luviana Kristianingtyas³,
Arindita Niatazya Novianti¹, Eka pramytha Hestianah⁴, Aswin Rafif Khairullah⁵

10.18805/ajdfr.DRF-408

ABSTRACT

The purpose of this study was to determine the effect of garlic extract (*Allium sativum*) in drinking water on the hematological profile of laying quail. In a completely randomized design (CRD), one hundred and twenty laying quail were randomly allocated to four treatments, with six replicates of five quail each. The treatment drink water are as follows, the control P0 (0%), 1% garlic extract (P1), 2% garlic extract (P2) and 4% garlic extract (P3). Feed and water were supplied *ad libitum*. The parameters measure were; the number of erythrocytes, hemoglobin, hematocrit, lymphocytes, monocytes and granulocytes. Blood samples from each quail were collected to analyze the hematological parameters. The result of hematological data showed that supplementation of garlic extract in drinking water had a significant effect ($P < 0.05$) on hemoglobin levels, but did not have a significant effect ($P > 0.05$) on erythrocyte, hematocrit, lymphocyte, monocyte and granulocyte levels. Meanwhile, the hemoglobin levels themselves showed that treatment P2 (2%) had a significant effect compared to other treatments. Based on clinical chemistry it shows that adding garlic extract with concentrations of 0% (control), 1%, 2% and 4% to drinking water does not have a significant effect ($P > 0.05$) on Aspartate transaminase (AST), Alanine aminotransferase (ALT), total cholesterol, triglycerides, BUN, creatinine and glucose. It can be concluded that the best treatment is giving 2% extract in drinking water which is able to improve the haematological profile of quail in terms of increasing hemoglobin levels.

Key words: Animal husbandry, Clinical chemistry, Garlic extract, Hematological, Laying quail.

One poultry species that is being developed and produced more commercially is the quail (*Coturnix coturnix Japonica*). Aside from meat, quails are highly productive eggs that yield 200-300 eggs per head annually (Akarikiya *et al.*, 2021). Quail eggs are just as nutritious as eggs from other birds, like hens (Wilson, 2017). There is a great deal of potential for poultry meat to bridge the gap between supply and demand for animal protein. Feed additives are added to chicken feed to raise the nutritional content of the components and improve broiler performance by accelerating growth and maximizing feed conversion. In order to increase productivity, broiler chicken rations have heavily utilized chemical feed additives including antibiotic growth promoters (AGP), yet these additives are well-known for causing bacterial resistance and having detrimental effects on consumer health (Paul *et al.*, 2022). This negatively affected profitability and performance of broiler which has triggered scientists to find alternative to AGPs for improving growth performance of broiler by optimizing their gut health (Junaid *et al.*, 2018 ; Saleem *et al.*, 2020). One alternative that can be applied to this problem is by adding feed additives.

One natural ingredient that has potential is garlic (*Allium sativum*). The biological properties of garlic, such as its hepatoprotective, antioxidant and antibacterial properties, have also been shown to support growth and strengthen the immune system (Karangiya *et al.*, 2016; Sangilimadan *et al.*, 2019). Chan *et al.* (2013) and Das

¹Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.

²Department of Veterinary Science, Faculty of Health Science, Medicine and Life Science, Universitas Airlangga, Jl. Wijaya Kusuma No. 113, Mojopanggung, Banyuwangi 68425, East Java, Indonesia.

³Program Study of Veterinary Medicine, Faculty of Health, Muhammadiyah University (UM) West Sumatra, Jl. Pasir Jambak No.4, Pasie Nan Tigo, Padang 25586, West Sumatra, Indonesia.

⁴Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.

⁵Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia.

Corresponding Author: Nanik Hidayatik, Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Email: nanik.h@fkh.unair.ac.id

How to cite this article: Hidayatik, N., Yuliani, G.A., Agustono, B., Kristianingtyas, L., Novianti, A.N., Hestianah, E.P. and Khairullah, A.R. (2024). Effect of Garlic Extract (*Allium sativum*) on Hematological and Clinical Chemistry Parameters of Laying Quail. Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DRF-408.

Submitted: 17-05-2024 **Accepted:** 22-06-2024 **Online:** 30-08-2024

et al. (2021) reported that *Allium sativum* dried powder improved antioxidant levels besides modulating oxidative stress. Garlic contains several organosulfur compounds, S-allylcysteine, allicin and alliin as phytochemicals. Fresh garlic contains alliin, a sulfoxide that is generated from cysteine. Garlic also contains discrete compartments containing the enzyme alliinase, which when crushed, transforms alline to allicin and gives garlic its characteristic smell as well as a number of pharmacological qualities (Venâncio *et al.*, 2017).

Broiler performance can be improved and the mortality rate can be reduced by adding garlic as a natural feed supplement (Kairalla *et al.*, 2022; Karangiya *et al.*, 2016; Puvaëa *et al.*, 2019). Results of earlier research on garlic and its derivatives in poultry generally indicated improvements in the hematological profile, immunological response, growth performance and antibacterial properties of the final product (Singh *et al.*, 2020). The hematological profile itself is usually used as an indicator that describes the health of the animal. By giving garlic to regular birds, mixed with the ration in the form of flour and added to the feed up to a level of 0.25%, it has been found to have a real effect on ration conversion and broiler performance index, but has no real effect on the blood profile of broilers (Javandel *et al.*, 2008). Therefore, in this research, development tests will be carried out to determine the appropriate application of garlic and or provide a significant effect on the hematological profile by drinking water.

Ethical approval

This research procedure has been declared ethically sound by the Research Ethics Commission of the Faculty of Veterinary Medicine, Airlangga University No. 1.KEH.102.06.2023 at Juni 23, 2023.

Time and location

The experiment was carried out at the Animal Laboratory Facilities of Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia from June to September 2023.

Materials

The materials used in this research include garlic powder from UPT Laboratorium Herbal Materia Medica Batu, disinfectants, mineral drinking water and 120 laying quail, commercial feed with a crude protein content of 20-22%, gloves, masks, syringes, alcohol, CMC-Na, 80 battery cages with dimensions of 60 cm × 50 cm × 30 cm each, equipped with a feed bowl, a drink cup and a drink tube for extract, digital scales with an accuracy of up to 1 gram, tools cage cleaner.

Extract preparation

Garlic (*Allium sativum*) extraction is carried out using a maceration process. *Allium sativum* extract was dissolved using distilled water in a ratio of 1:2 with for 48 hours. Filtration process was carried out to obtain the result in the form of macerate. The macerate was evaporated using a

rotary evaporator at a temperature of 50°C with a speed of 50 rpm. Next, the extraction results were used further for *in vivo* testing.

Preparation

Disinfection of the cage is carried out before the quail arrive. The quails were acclimatized for 14 days. On the first until third days of arrived, quail were given drinking water mixed with 3% sugar and poultry vitamin for reducing the stress. Feed and drink were provided *ad libitum* during the acclimatization.

Study design

This study used a Completely Randomized Design (CRD), one hundred and twenty laying quail were randomly allocated to four treatments, with six replicates of five quail each.

Treatment is as follows:

- P0: Control/without adding *Allium sativum* extract in the drinking water.
- P1: Drinking water added by *Allium sativum* extract with 0.5 gr/50 ml water (1%).
- P2: Drinking water added by *Allium sativum* extract with 1 gr/50 ml water (2%).
- P3: Drinking water added by *Allium sativum* extract with 2 gr/50 ml water (4%).

This treatment above was carried out for 28 days. The extract was applied at one hour before the feeding time. The non-chlorine drinking water given *ad-libitum* after the extract was finished.

Blood collection and haematological profil analysis

Quail blood was collected using a 1 cc syringe via the brachial vein as much as 1-2 ml, then the blood was put into a blood tube treated with anticoagulant. Next, the blood sample was analyzed for a complete blood profile using a hematology analyzer.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA). If there are significant differences between treatments, Duncan's multiple range test is carried out with a significant differences ($p < 0.05$).

The effect of garlic extract on the hematological profile of laying quail consisting of the number of erythrocytes, hemoglobin levels, hematocrit levels, lymphocytes, monocytes and granulocytes in each treatment is presented in Table 1.

Based on statistical results, it shows that the addition of garlic extract can provide significantly different results ($P < 0.05$) on hemoglobin levels, but not significantly different ($P > 0.05$) on erythrocyte values, hematocrit levels, lymphocytes, monocytes and granulocytes. Of the several treatments on hemoglobin levels, treatment P2 (2% garlic extract) was the treatment that gave significant results compared to other treatments, in the sense that a concentration of 2% garlic extract in the drinking water of laying quail was able to have a real effect on hemoglobin levels.

The effect of giving garlic extract on the blood chemical values of laying quails consisting of Aspartate transaminase (AST), Alanine aminotransferase (ALT), total cholesterol, triglycerides, Blood Urea Nitrogen/BUN, creatinine and Glucose in each treatment is presented in Table 2.

Based on statistical results, it shows that adding garlic extract with concentrations of 0% (control), 1%, 2% and 4% to drinking water does not have a significant effect ($P>0.05$) on Aspartate transaminase (AST), Alanine aminotransferase (ALT), total cholesterol, triglycerides, BUN, creatinine and glucose.

The number of erythrocytes is an indicator that describes health status and the adequacy of nutrition in the body (Shittuet *et al.*, 2016). Based on the results of data analysis, the addition of garlic extract did not have a significant effect ($P>0.05$) on the number of erythrocytes in laying quail. The highest average number of erythrocytes was in P2, namely $3.65 \times 10^6/\text{mm}^3$, followed by P1 and P3, namely $3.38 \times 10^6/\text{mm}^3$, while the lowest number of erythrocytes was in the control treatment at $3.3 \times 10^6/\text{mm}^3$. From this value, the erythrocyte value is still within the normal value range, namely $2.5\text{-}3.5 \times 10^6/\text{mm}^3$ and is able to be maintained at normal levels. There are several things that influence the high and low values of erythrocytes, including because the feed nutrition required by each individual laying quail is different or because of environmental factors, including environmental temperature (Negedu *et al.*, 2023). In another hand, the number of erythrocytes can also be influenced by feed quality, phase/age and sex (Golbeck *et al.*, 2019).

Hemoglobin is a form of protein that plays an important role in binding and distributing oxygen to and from the lungs and throughout the body. Hemoglobin levels are also responsible for binding and transporting CO_2 (Straková *et al.*, 2010). The administration of garlic extract in this study had a significant effect ($P<0.05$) on hemoglobin levels, as evidenced by the increase in hemoglobin levels compared to normal levels, namely 7.0-13.0 g/dL. From several treatments that have been carried out, it was found that treatment at P2 had a significant effect on increasing hemoglobin levels, namely 17.28 g/dL. Based on previous research, the application of garlic can be mixed into feed in the form of dry flour. Javandelet *et al.* (2008) stated that the addition of garlic flour up to a level of 0.25% in broiler rations had a significant effect on ration conversion and broiler performance index. However, it has no real effect on the broiler's blood profile. Meanwhile, research conducted by Olumide and Odunowo (2019) showed that adding 0.8% to feed had an insignificant effect ($P>0.05$) on the hematological response in broiler chickens, but succeeded in increasing blood cholesterol levels. Increased of hemoglobin levels in this study is probably due to the effect of the antioxidant content in garlic which can fight free radicals so that the number of red blood cells can be maintained and their function in binding oxygen runs well so that the blood is able to circulate oxygen and maintain hemoglobin levels in the blood. This is also in accordance with the opinion of Revin *et al.* (2019) that by increasing antioxidants, the body can reduce and prevent oxidative stress which affects hemoglobin levels.

Table 1: Effect of garlic supplementation on hematological parameters.

Parameters	Treatments			
	P0 (Control)	P1 (1%)	P2 (2%)	P3 (4%)
Erythrocytes ($10^6/\text{mm}^3$)	3.3	3.38	3.65	3.38
Hemoglobin (g/dL)	15.85 ^b	15.72 ^b	17.28 ^a	15.6 ^b
Hematocrit (%)	45.57	44.1	48.35	44.3
Lymphocyte (%)	77.83	78.5	76.33	77.8
Monocyte (%)	17.5	17	18.16	17.2
Granulocytes (%)	4.66	4.5	5.5	5

Note: ^{ab}Values within the same row followed by different subscripts differ significantly ($P<0.05$).

Table 2: Effect of garlic supplementation on biochemical parameters.

Parameters	Treatments			
	P0 (Control)	P1 (1%)	P2 (2%)	P3 (4%)
AST (U/L)	261.17	232.67	258.17	245.33
ALT (U/L)	7.3	3.3	6.3	5
Total cholesterol (mg/dL)	144.83	124.5	117	150.33
Triglycerides (mg/dL)	901.17	800.33	740.33	1110
BUN (mg/dL)	2.25	2.17	2.5	4.3
Creatinine (mg/dL)	0.3	0.2	0.4	0.2
Serum glucose (mg/dL)	210.67	234	251.67	253.67

Also found by Makwana *et al.* (2015); Chitra (2020) and Hayat *et al.* (2022), it was stated that adding garlic to broiler chicken feed could increase growth significantly. This increase was associated with the antimicrobial and antioxidant performance of garlic. Therefore, garlic is considered as an alternative as a growth promoter in poultry because it can improve the performance of intestinal villi which causes efficient absorption of nutrients. Vargas *et al.* (2022), the present work support the use of purple garlic from Las Pedroneras, Spain (standardized commercial product) supplemented in the feed of broiler in a daily concentration of 0.2-0.3% as a natural alternative and viable tool in broiler for growth promotion.

Olumide and Odunowo (2019) also stated that with the addition of garlic the Packed Cell Volume (PCV) value which is involved in transporting oxygen and absorbing nutrients also increases so that it can prevent anemia. Apart from that, PCV and hemoglobin are also positively correlated with nutritional status so they can have a good effect on growth. The hematocrit value is the percentage of blood volume that contains red blood cells. The hematocrit value in this study was also high compared to the normal value of between 30.2-32.3%. After adding 2% garlic extract, treatment P2 had the highest value, increasing to 48.35%; P3 and P1 followed with 44.3% and 44.1%, respectively. This also correlates with erythrocyte and hemoglobin levels themselves. According to Jamil *et al.* (2015) that high and low concentrations of hematocrit values are positively correlated with erythrocyte and hemoglobin values.

The effect of adding garlic extract on the values of lymphocytes, monocytes and granulocytes had no significant effect ($P>0.05$) on laying quail. In this study, although the three of them did not have a real effect, the addition of garlic extract was enough to maintain the value of the three so that they did not have a negative effect in this case.

Based on the clinical chemistry analysis, there was no significant effect between treatments, the AST, ALT, total cholesterol and triglyceride values, but adding garlic extract to drinking water caused a decrease thus parameters when compared to the control.

Previous study provided significant results with the addition of garlic powder in concentrations of 5 g/kg, 10 g/kg and 15 g/kg feed on cholesterol and triglyceride levels at 16 weeks post-treatment (Udeh *et al.*, 2018). It is anticipated that the feed's high fiber content will be the source of both this value's increase and decline. High fiber in feed can increase bile excretion and thus cholesterol in the blood will decrease (Soliman, 2019). The decrease in quail blood cholesterol can also be caused by substances such as carvacrol and thymol found in plants such as garlic and thyme (Gholami-Ahangaran *et al.*, 2022).

It was also found in research that with a concentration of 0.5%, 1%, 1.5% and 2% garlic powder in the feed, it showed that the total protein content increased and serum glucose, triglycerides, total cholesterol and creatinine

decreased (Dehkordi *et al.*, 2010). From the results previously described, the addition of garlic is mostly given in the form of dry powder and added to the feed in very varying concentrations. With different application applications, it is likely that they will also provide different results. In addition, a number of other variables, such as gender, body weight, amount of food consumed, age and environment, also have an impact.

CONCLUSION

Garlic extract has potential as an antioxidant in laying quail. The addition of garlic extract to the drinking water of laying quail at 2% (Drinking water added by *Allium sativum* extract with 1 gr/50 ml water) which is given one hour before feeding via a drinking tube, this is expected to be the drinking water that has been added the garlic extract is completely used up (2-3 hours) and is not wasted during the process so it was able to have a significant effect on improving the hematological profile, especially hemoglobin levels. Meanwhile, the other parameters of hematological and clinical chemistry levels, can be said that adding garlic extract to drinking water does not have a bad impact on the body and health of laying quail.

Because this research is preliminary research, it is hoped that there will be further research to be able to find a concentration that is truly capable of providing a significant effect in providing beneficial effects on hematological and blood chemistry levels in laying quail so that a standard concentration can be used for administration via drinking water, which does not yet exist. previous research conducted.

ACKNOWLEDGEMENT

The author would like to thank Institute For Research and Community Service (LPPM) Universitas Airlangga and Faculty of Veterinary Medicine Universitas Airlangga for the providing the Airlangga Research Fund Universitas Airlangga 2023 with contract number 1288/UN3.1.6/PT/2023.

Conflict of interest

The authors declare that they have no competing interests.

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