Giving bidara leaf extract (*Ziziphus mauritiana*) through drinking water as an alternative antioxidant against quail haematological

Giving bidara leaf extract (*Ziziphus mauritiana*) through drinking water as an alternative antioxidant against quail haematological

S Supratman[1], S Purwanti[2] and D P Rahardja[2]

[1]Postgraduate Program, Animal Science and Technology, Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia 90245
[2]Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia 90245

Email: sripurwanti@unhas.ac.id

Abstract. Bidara leaves (*Ziziphus mauritiana*) contain quercetin 3-O-rhamnoglucoside 7-O-rhamnoside which is a major flavonoid compound that has the potential as an antioxidant. This study was aimed to determine the effect of giving bidara leaf extract as an alternative antioxidant on the haematological status of quail. This study used 160 quails aged 9 days that were reared for 42 days. This study used a completely randomized design, which consisted of 5 treatments and 4 replications. Using the experimental arrangement: P0 (without vitamin C and bidara leaf extract); P1(vitamin C); P2 (0.20 mL bidara leaf extract); P3 (0.24 mL bidara leaf extract) and P4 (0.28 mL bidara leaf extract). Parameters measured were hematocrit, haemoglobin, erythrocyte and leukocyte. The result showed that erythrocytes and leucocytes had no significant effect ($P>0.05$) but had a significant effect ($P<0.05$) on haemoglobin and hematocrit. The highest haemoglobin level was at P4 while the lowest haemoglobin level was at P0. The treatment of P3 (0.24 mL of bidara leaf extract) can increase the levels of quail hematocrit. Therefore, it can be concluded that the higher level of giving bidara leaf extract, the higher of haemoglobin level in quails and giving bidara leaf extract at P3 (0.24 mL) was the best alternative to an antioxidant in quails.

1. Introduction

Quail (*Coturnix-coturnix japonica*) has great potential to be further developed as an alternative cheap source of animal protein. The data of Animal Husbandry and Animal Health that the quail population in 2017 was 14,427 million or an increase of 2.21% from the previous year of 14,108 million [1]. This shows that quail has a role as a provider of animal protein intake which is very meaningful for the community. However, quail has an immune system that is susceptible to diseases caused by free radicals, so an alternative addition of antioxidants is needed to improve performance and body haematology in quail, as well as to see the effect on the digestive organs.

Antioxidants are compounds that can counteract the negative effects of oxidants in the body such as Reactive Oxygen Species (ROS) and other free radicals. Antioxidants are in the form of vitamins, minerals, or a type of nutrient that plays a role in maintaining and repairing cells damaged by free radicals. Meanwhile, free radicals can be caused by pollution, cigarette smoke and chemical compounds. Antioxidants can come from the body and the intake of nutrients contained in a food/feed. One of the natural plants that have high antioxidants is bidara leaves.

The leaves of bidara (*Ziziphus mauritiana*) have long been used to help maintain a healthy lifestyle. Bidara leaves have the role of providing antibacterial activity against several pathogenic bacteria, namely *Escherichia coli*. Bidara leaves contain quercetin 3-O-rhamnoglucoside 7-O-rhamnoside which is the main flavonoid compound in all parts of the plant that has potential as an antioxidant. The highest flavonoid content was found in leaves (0.66%). Flavonoids can be used as immunomodulators in the immune system to increase body resistance in chicken [2]. This research was previously carried out by Supratman [3] using bidara leaf extract at a level of 0.24 mL with 120 ppm. The results showed that the antioxidant at the level of P4: 0.24 mL was 95.79%. This value indicated that the leaves of bidara (*Ziziphus mauritiana*) had the potential as a strong antioxidant. Therefore the potential possessed by bidara leaves, the become background for researching studying the administration of bidara leaf extract (*Ziziphus mauritiana*) through drinking water as an antioxidant alternative to the performance, haematology, and digestive organs of quail.

2. Research methods

This research was conducted in July-October 2020 at the Non-Ruminant Laboratory of the Faculty of Animal Science, Universitas Hasanuddin. The material used in this study was 160 female quail aged 9 days consisting of 80 males and 80 females obtained from breeders in Gowa. Each quail is placed into a battery cage measuring 90 x 60 x 30 cm made of ram wire and bamboo. Each cage unit consists of 8 quail, equipped with a place to feed, drinking water and a 20-watt incandescent lamp. The equipment used was stationery, Weiheng digital scale by load power 500 g, sensitivity 0.01 g, scissors, gloves, hand sprayer, syringe, ruler, and EDTA tube.

The making of bidara leaf extract used the infusion method, namely the water-solvent extraction method. During the infusion process, the water solvent temperature must reach 90ºC for 15 minutes. The ratio of the weight of the material and water is 1:10, meaning that if the weight of the material is 100 grams, the volume of water as a solvent is 1000 mL. The way this is done was the ingredients are heated in a saucepan with enough water for 15 minutes starting from 90ºC, stirring occasionally. Filter it while hot through a flannel cloth, add enough hot water through the dregs until the desired volume was obtained. Filtering was done after the cold.

The feed ingredients consisted of a mixture of feed ingredients, namely: yellow corn, SBM, MBM, CGM, palm olein, essential amino acids, essential minerals, vitamins and premix. The composition and nutritional content of the feed ingredients used in the study are presented in table 1.

Table 1. Quail feed nutrient (commercial feed) content during research.

Feed Nutrient Content	Value
Crude Protein	20
Extract Ether	45
Crude fiber	5
P	0.5
Ca	0.65
Ash	8
Enzymes	+

The design used in this study was a completely randomized design consisting of five treatments and four replications, using the experimental arrangement: P0 (rations without vitamin C and leaf extract of bidara); P1 (vitamin C); P2 (0.20 mL of bidara leaf extract; P3 (0.24 mL of bidara leaf extract); P4 (0.28 mL of bidara leaf extract). Testing of experimental data was carried out using ANOVA analysis. Duncan continued the test to determine the significant difference between treatments if it was below the significant level ($P<0.05$).

Haematocrit data was collected using anticoagulant and put into a microhematocrit pipette about 6/7 parts of the pipette. Cover the end of the entry of blood with a special cover or by using wax (seal). The pipette was placed in a microhematocrit centrifuge. Then messed around with a speed of 10,000 rpm for 5 minutes. Then the hematocrit value obtained was read on a special reading device (microhematocrit reader) [4]. Haemoglobin data collection was carried out by using a hemometer. Blood with an anticoagulant was sucked with a Sahli pipette until it was just at the 20 mm^3 marks. The outside of the pipette was cleaned with tissue paper. Blood was immediately carefully inserted into the hemometer tube containing 0.1 N HCl without causing air bubbles. Before removing, the pipette was rinsed by sucking and blowing the HCl in the tube several times. The outside of the pipette was also rinsed with a few drops of distilled water. Wait 10 minutes for the formation of hematin acid. After hematin acid was formed, this acid was then diluted with distilled water drop by drop while stirring until the color was the same as the brown color in the standard glass. Miniscus of the solution was read on a 9% scale [4].

Hematocrit data were collected using the Burker space method. The differential count of blood leukocytes was determined by counting the smear preparations using a light microscope with an immersion lens. The coverslip technique was applied when preparing the blood smear [4]. Meanwhile, the leucocyte data collection, namely the counting room was prepared, the cover glass was placed above the counting room so that it covered the counting area. Blood that had been given an anticoagulant was sucked with an erythrocyte pipette up to the 0.5 marks. If it exceeded the limit, the blood was drained by touching the tip of the dropper with the tip of the finger. The outside of the pipette was removed with tissue paper. Immediately the Hayem diluent solution was sucked up to the 101 marks. During inhalation, the pipette must be circled along its long axis so that the blood with Hayem's solution was well mixed. The two tips of the pipette were covered with the thumb and middle finger and then shaken in a perpendicular motion to the long axis for two minutes. Diluted the diluents which were inside the capillaries and those which did not contain blood was removed by dropping three drops. The blood solution was entered into the counting room by placing the tip of the pipette on the edge of the closing glass. Because of the capillary power, the blood solution would flow into the cover glass and the counting room, the blood solution should not be too much.

The counting chamber which already contained the blood solution was placed under a microscope and the count was done using a 45x objective lens. The calculation was carried out as follows: count the number of erythrocytes contained in the 5 central planes with an area of 1/25 mm^2 each, the cells offending the left and lower boundary lines were not counted, the method of counting must be systematic to avoid one cell being counted more than one time. The calculation was carried out as follows: Suppose the number of erythrocytes in the five fields was N, the total volume of the five fields is 5/250 mm^3. Therefore, for every mm^3 of blood there is: (1: 5/250) x N = (250: 5) N = 50 N erythrocytes. With 200 dilutions, the number of erythrocytes per mm^3 of blood is 50 N x 200 = 10,000 N. For the calculation to be accurate, two counts were carried out in both counting rooms. The error using this method was about 7.8% [4].

3. Results and discussion

The results of observations on the hematocrit, haemoglobin, erythrocytes and leukocytes in quail given different levels of bidara leaf extract (*Ziziphus mauritiana*) could be presented in table 2.

The effect of giving bidara leaf extract as an alternative antioxidant for quail could be determined by observing the haematological parameters. Haematologist has an important role in determining the health status of quail. The results of statistical analysis showed that giving bidara leaf extract to quail drinking water at different levels had no significant effect ($P>0.05$) on erythrocytes and leucocytes, but had a significant effect ($P<0.05$) on quail hematocrit and haemoglobin.

Table 2. Haematological status of quail blood given different levels of bidara leaf extract (*Ziziphus mauritiana*).

Parameters	Treatment			
	Hematocrit (%)	Hemoglobin (g/dl)	Erythrocytes (10^6/mm^3)	Leukocytes (10^3/ mm^3)
P0	32.50±6.58[a]	18.95±0.94[a]	4.61±0.99	41.11±6.82
P1	37.33±2.17[ab]	18.95±0.28[a]	3.99±0.23	35.40±15.54
P2	37.87±2.78[ab]	20.45±0.49[b]	4.82±1.31	32.75±17.56
P3	39.50±0.79[b]	21.45±1.30[b]	5.80±1.79	31.52±3.89
P4	38.76±3.90[b]	21.87±1.14[b]	5.59±1.40	34.38±2.97
N	37	12.3	1.25-4.50	20-30

[ab]Different superscripts on the same colom showed significant differences (P<0.05).

3.1. Hematocrit

Based on the results of statistical analysis, giving bidara leaf extract had a significant effect (P<0.05) on the hematocrit levels of quail. The highest level of hematocrit was found in treatment P3, namely 39.50%, while the lowest level of hematocrit was found in treatment P0, namely 32.50%. According to levels of hematocrit are influenced by external factors including ration, water consumption, environmental and internal temperature including age, nation, sex and livestock activity [5]. Also, if there is a decrease in hematocrit due to a deficiency of amino acids in the feed, while the increase in hematocrit is due to dehydration so that the ratio of erythrocytes to blood plasma is above normal [6]. The normal hematocrit in quail according to is 37% has an important role in determining the health status of quail [7]. The health of quail can be seen from the level of hematocrit which is a measuring tool for blood health because blood is a medium for the formation of antibodies.

Hematocrit is the percentage of blood volume that contains red blood cells. The percentage of hematocrit is the percentage of erythrocytes in the entire blood volume, so the increase in the percentage of hematocrit is directly proportional to the increase in the number of erythrocytes. An increase in the value of hematocrit, haemoglobin, and the number of erythrocytes above the normal range can also be caused by the occurrence of erythrocytosis. The number of erythrocytes is closely related to the level of stress in livestock which is influenced by environmental conditions and the maintenance system. Factors that influence the value of the hematocrit are sex, species and the number of red blood cells. Activity and pathological conditions, as well as altitude also affect the value of the hematocrit.

3.2. Hemoglobin

Bidara leaf extract had a significant effect (P<0.05) on the haemoglobin level of quails. The value of hemoglobin levels shows that P0(18.95 g/dl), P1 (18.95 g/dl), P2 (20.45 g/dl), P3 (21.45 g/dl) and P4 (21.87 g/dl). From the existing data, it could be seen that the addition of bidara leaf extract in quail drinking water could increase the haemoglobin level of quails. The higher the level of bidara leaf extract, the higher the haemoglobin level in the quail. Bidara leaf extract contains flavonoids that accelerate the absorption of feed nutrients and absorption of Fe nutrients which function to form haemoglobin. Explained that haemoglobin synthesis begins with the proeritoblast phase and then continues in the reticulocyte phase in the bone marrow [9]. The basic chemical stage for the formation of haemoglobin, namely succinyl CoA which is formed in the Krebs cycle binds with glycine to form pyrrole compounds which combine to form protoporphyrin compounds. Then these compounds bind to iron using the help of the enzyme ferrochelatase to form a heme molecule. Each heme molecule combines with a long polypeptide chain (globin) to form a haemoglobin subunit. Haemoglobin levels also indicate the adequacy of nutrients in the feed, namely meeting the protein needs [10]. Normal haemoglobin levels indicate sufficient oxygen to be circulated to all body tissues.

3.3. Erythrocytes

Results of statistical analysis the average erythrocyte values from the highest to the lowest in the quail erythrocyte count were P3 ($5.80x10^6/mm^3$), P4 ($5.59x10^6/mm^3$), P3 ($4.82x10^6/mm^3$), P1 ($4.61x10^6/mm^3$) and P2 ($3.99x10^6/mm^3$). From the available data, it could be seen that the addition of bidara leaf extract treatment in quail drinking water did not affect quail erythrocytes. The increase and decrease in the total number of erythrocytes are influenced by the increase in age and mass of blood cells as well as influenced by gender and environmental factors [11].

In the research conducted, the temperature and air temperature conditions were unstable due to the construction and type of cage used without the help of a fan so that stress and heat stress occurs in the quails. This results in the performance of erythrocytes, which function to transport oxygen from the lungs to various body tissues, not working optimally. A higher erythrocyte count is an indicator of the availability of protein and amino acids needed in the formation of optimal erythrocytes so that they can support erythrocyte formation effectively and efficiently [12].

A low number of erythrocytes is an indicator that the availability of oxygen for the body's metabolic processes is not yet as needed. Apart from the suboptimal availability of oxygen, the low number of erythrocytes can also be caused by the low availability of protein and amino acids that play a role in the process of erythrocyte formation. This condition ultimately results in a low erythrocyte count as evidenced in this study. Campbell and Ellis (2012) stated that a low erythrocyte count can be an indication of anaemia, while a high erythrocyte count is an indication of polysetamia. Furthermore, it was reported that the high and a low number of erythrocytes were influenced by age, individual activity, nutrient content of the feed, altitude and ambient temperature [13].

3.4. Leukocytes

Statistical analysis result giving bidara leaf extract had no significant effect ($P>0.05$) on the levels of quail leukocytes. From the data obtained in the table shows that P0 ($41.11x10^3/mm^3$), P1 ($35.40x10^3/mm^3$), P2 ($32.75x10^3/mm^3$), P3 ($31.52x10^3/mm^3$) and P4 ($34.38x10^3/mm^3$). The normal range of quail leukocyte counts is 20–40 thousand mm^3 [14]. The results of the data obtained indicate that the addition of bidara leaf extract P2 ($32,75x10^3/mm^3$), P3 ($31.52x10^3/mm^3$) and P4 ($34.38x10^3/mm^3$) could increase quail leukocytes, this is because bidara leaves have a role to provide activity antibacterial against several pathogenic bacteria, namely *Escherichia coli*. Flavonoids as immunomodulators in the immune system increase body resistance [15].

The data that shown that the provision of vitamin C, namely P1 is $35.40x10^3/mm^3$ also shows that the leukocytes are still in the normal range, this indicated that giving vitamin C to quails that were exposed to heat could prevent oxidative stress. Soegondo et al (2005) the increased need for vitamin C needs to be balanced with the addition of vitamin C from outside the body so that the impact of stress can be overcome [16].

4. Conclusion

The result showed that erythrocytes and leucocytes had no significant effect on haemoglobin and hematocrit. The h higher the level of giving bidara leaf extract, the higher of haemoglobin level in quails and giving bidara leaf extract at 0.24 mL was the best alternative to an antioxidant in quails.

References

[1] Dirjen Peternakan dan Kesehatan Hewan 2017 *Statistik Peternakan dan Kesehatan Hewan Kementerian Pertanian RI* (Jakarta: Dirjen Peternakan dan Kesehatan Hewan)

[2] Erin K, Sasmito M and Rifa'i M 2013 Aktivitas imunomodulator polyscias obtuse terhadap sistem imunitas pada bone marrow broiler setelah pemberian *Salmonella typhimurium J. Experimen. Life Sci.* **3** 25-29

[3] Supratman S 2018 *Uji Antioksidan Daun Bidara (Ziziphus mauritiana) Sebagai Alternatif Feed Additive Pada Unggas* Skripsi (Makassar: Universitas Hasanuddin)
[4] Dharmawan N S 2002 *Pengantar Patologi Klinik Veteriner (Hematologi Klinik)* (Denpasar: Pelawa Sari)
[5] Schalm, Jain N C and Carrol E J 1986 *Veterinary Hematology* (Jakarta: Penebar Swadaya)
[6] Frandson R D 1993 *Darah dan Cairan Tubuh Lainnya* (Gajah Mada University Press)
[7] Sturkie P D and Griminger P 1976 *Blood: physical characteristics, formed elements, haemoglobin and coagulation* (New York: Springer-Verlag)
[8] Ganong W 1996 *Fisiologi Kedokteran* (Jakarta: EGC)
[9] Guyton A C dan Hall J E 1997 *Buku Ajar Fisiologi Kedokteran* (Jakarta: EGC)
[10] Olugbemi T S, Mutayoba S K and Lekule F P Effect of moringa *(Moringa oleifera*) inclusion in cassava-based diets fed to broiler chickens *Int. J. Poult. Sci*. **9** 363-67
[11] Adeyemo G O, Ologhobo A D and Adebiyi O A 2010 The effect of graded levels of dietary methionine on the haematology and serum biochemistry of broilers *Int. J. Poult. Sci*. **9** 158-61
[12] Hilmi M 2015 *Penambahan Piperin sebagai Imbuhan Pakan Fitogenik terhadap Performa, Metabolisme Lemak dan Hematologi Puyuh Petelur* (*Coturnix coturnix japonica*) Tesis (Bogor: IPB)
[13] Campbell T W and Ellis C K 2012 *Avian and Exotic Animal Hematology* (Colorado: Wiley Blackwell Publishing)
[14] Sturkie P D and P Griminger 1976 *Blood: physical characteristics, formed elements, hemoglobin and coagulation* (New York: Springer-Verlag)
[15] Erin K, Sasmito M and Rifa'I M 2013 Aktivitas imunomodulator polyscias obtuse terhadap sistem imunitas pada bone marrow broiler setelah pemberian *Salmonella typhimurium J. Experimen. Life Sci.* **3** 25-9
[16] Soegondo S, Soewondo P and Subekti I 2005 *Penata Laksanaan Diabetes mellitus Terpadu, Sebagai Panduan Penatalaksanaan Diabetes mellitus bagi Dokter Maupun Edukato*r (Jakarta: Universitas Indonesia)