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Effects of Burahol Fruit Flour (*Stelechocarpus burahol*) Supplementation on Blood Plasma Biochemistry and Growth Performance of Water-Restricted Cihateup Ducks

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Abstract. This study aims to determine the effect of providing burahol fruit flour (*Stelechocarpus burahol*) in feed on the blood biochemical profile and performance of Cihateup Ducks raised without access to water. Ducks were raised in the Jaya Organik livestock group pen, Ciseupang, Kasomalang, Subang from 2 to 5 months of age. This study used an unpaired T-test. The number of Cihateup Ducks raised was 300 with 150 ducks per treatment. Ducks were randomly assigned to two treatments, namely treatment P0 in the form of rations provided without burahol fruit flour and treatment P1 in the form of rations provided with feed additives burahol fruit flour combined with flavonoids, methionine, linoleic acid and allicin from garlic essential oil as much as 15 g/kg ration. The research data showed that the administration of burahol fruit flour had a significant difference ($P<0.05$) on the biochemical profile of Cihateup ducks, namely uric acid 19.67 mg/dL, urea 2.19 mg/dL, albumin 2.75 mg/dL, total protein 8.23 mg/dL, glucose 217.18 mg/dL, triglycerides 101.89%, RNA 7.05%, and MDA 1.06 nm/M-1cm-1, as well as the performance of Cihateup ducks, namely an average egg production of 88.97%, an average egg weight of 84.92%, an average shell thickness of 3.15 mm, and an average hatchability of Cihateup ducks of 88.66%.
Keywords: blood plasma, burahol fruit flour, cihateup duck, performance.

1 Introduction

The Cihateup duck is a local West Javanese livestock that has undergone a domestication process. The Cihateup duck originates from Cihateup Hamlet, Sukanagalih Village, Rajapolah District, Tasikmalaya Regency. The Cihateup duck has been certified as a native West Javanese livestock genetic resource based on the Decree of the Ministry of Agriculture No. 425/KPTS/SR.120/3/2014. The Cihateup duck is a type of local Indonesian poultry

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germplasm that has significant potential for development as a meat and egg producer [1]. Cihateup ducks have high productivity, averaging 275 eggs per bird per year, with an adult mortality rate of around 2-5%.

Ducks naturally require access to water for drinking, swimming, and grooming to maintain optimal performance and health, as they are waterfowl [2]. However, in intensive farming systems or in areas with limited water access, this water requirement often becomes a major obstacle, causing stress in the chickens and potentially reducing production. Water restriction can trigger complex physiological and biochemical responses in ducks. Dehydration stress is thought to disrupt metabolic balance, which is then reflected in changes in the biochemical profile of blood plasma. Observed parameters include levels of uric acid, urea, albumin, total protein, glucose, triglycerides, RNA, MDA, Hens-Day Production (HDP), egg weight, and shell thickness, which can be important indicators of immunity, energy metabolism, and organ function in response to stress [3,4]. These changes in biochemical profiles ultimately affect duck performance, including weight gain, feed consumption, and feed utilization efficiency.

Population growth requires innovative strategies to mitigate the negative impacts of water restriction. Previous research has shown that several active substances from natural ingredients have the potential to enhance immunity and improve blood profiles [5]. Burahol fruit flour (*Stelechocarpus burahol*) is a feed additive known to be rich in fiber, antioxidants, and bioactive compounds that can maintain fluid balance and biochemical status in ducks under conditions of limited water access. Burahol fruit flour contains flavonoids and tannins, which work together with methionine and linoleic acid to increase the efficiency of protein metabolism. Phenolic and flavonoid content plays a role in preventing damage caused by free radicals with concentrations of 73.09% and 30.99%, respectively [6]. Other studies have shown that the flavonoid content in burahol leaves plays a role in inhibiting bacterial growth. The tannins in burahol can also capture excess protein, preventing initial damage caused by bacteria in the rumen or intestine, and ensuring protein is available for absorption in the small intestine. Burahol leaf extract given to quail can improve ration efficiency due to its flavonoid content, resulting in lower conversion and higher productivity. This study focused on internal organ weight, which indirectly helps increase overall body weight.

Based on this background, this study was conducted to analyze the impact of burahol fruit flour administration on the blood plasma biochemical profile and performance of Cihateup ducks raised without access to water. The results of this research are expected to provide practical and locally based methods for caring for ducks more efficiently, especially in areas experiencing water shortages, while also helping to preserve and improve the potential of the Cihateup Duck as a quality local poultry species.

2 Materials and methods

1.1 Materials

The research materials used are: 1. Cihateup ducks were raised for 3 months, namely from the age of 2 to 5 months. The ducks used were ducks from the Jaya Organik livestock group, Ciseupang, Kasomalang, Subang with an average weight of 1.8 ± 0.1 kg, 2) Burahol Fruit Flour (Stelocarpus burahol); 3) Cages and cage equipment; and 4) Basal feed using commercial basal feed produced by PT. Japfa Comfeed Tbk.

1.2 Research Methods

The research was conducted at the Jaya Organik livestock group, Ciseupang, Kasomalang, Subang.This study used spectrophotometric techniques following measurement procedures based on the KIT Biolabo analysis protocol. Blood plasma biochemical analysis was conducted at the Animal Physiology and Biochemistry Laboratory, Faculty of Animal Science, Padjadjaran University.

1.3 Research Design

The ducks were divided into two treatment groups, namely P1 (ducks without feed additives) and P2 (ducks with feed additives (a combination of flavonoids, methionine, linoleate). Each treatment group consisted of 150 ducks. Each experimental livestock was given a number on its wing (wing tag) according to the treatment to facilitate observation and data collection during maintenance.

1.4 Process of Making Burahol Fruit Flour *(Stelocarpus burahol)*

The selected fruit was at an optimal level of ripeness, with a brownish color and a strong, distinctive aroma. Damaged, rotten, or moldy fruit was discarded to ensure the quality of the resulting flour. Burahol fruit was washed with clean water to remove dirt and dust from its surface. After washing, the fruit is separated into flesh and seeds. The flesh was then cut into small pieces to speed up the drying process. Drying was carried out in an oven at 50 to 60 degrees Celsius for 8 to 10 hours. Once dry, the burahol fruit pieces are crushed using a hammer mill to form a fine powder. The crushed flour was then sieved through a 60-mesh sieve to ensure an even particle size. Any remaining coarse flour is further crushed to achieve a fine texture that meets feed research standards. The refined burahol flour was stored in a tightly closed container, such as a glass jar or airtight plastic container, and stored in a dry place protected from sunlight to prevent oxidation or quality degradation. This flour is ready to be used as a feed additive in animal feed formulations.

1.5 Observed Variables

The variables observed in this study were biochemical profiles, including uric acid, urea, albumin, total protein, glucose, triglycerides, RNA, MDA, as well as livestock performance, including egg production percentage, egg weight, shell thickness and hatchability percentage.

1.6 Statistical Analysis

The research data will be analyzed using the unpaired T-test.

2 Results and discussion

The effect of providing burahol fruit flour in rations on the biochemical profile of Cihateup duck blood plasma based on the research results can be seen in Table 1

2.1 Effect fruit flour (*Stelechocarpus burahol*) in feed on blood plasma

The study summarizes the effect of giving burahol fruit flour *(Stelechocarpus burahol)* in feed on blood plasma, namely uric acid, urea (NH_3), albumin, total protein, glucose, triglycerides, RNA and MDA, as well as the performance of Cihateup ducks including the average percentage of egg production, average egg weight, average shell thickness. and percentage of hatchability in Table 1.

Table 1. Biochemical Profile of Cihateup Duck Blood Plasma

Response	Group of Ducks	
	P0	P1
Uric acid (mg/dL)	21.45^{a}	19.67^{b}
Urea-NH3 (mg/dL)	4.71^{a}	2.19^{b}
Albumin (mg/dL)	5.26^{a}	2.75^{b}
Total protein (mg/dL)	4.34^{a}	8.23b
Glucose (mg/dL)	214.89^{a}	217.18^{a}
Triglycerides (mg/dL)	118.64^{a}	101.89^{b}
RNA (%)	34.83^{a}	7.05^{b}
MDA (nm/M-1cm-1)	4.18^{a}	1.06^{b}

The average followed by a superscript of different alphabetic elements shows a significant difference (P<0.05)
Information :
P0: Without giving feed additives
P1: Addition of feed additive for burahol flour (*Stelechocarpus burahol*).

Based on the results of this study, it can be seen that the average value of the uric acid profile decreased before and after administration of burahol fruit flour, namely from 21.45 mg/dL to 19.67 mg/dL. Burahol flour is used as a traditional medicine to lower cholesterol and uric acid levels [7]. Burahol flour contains chemical compounds in the form of polyphenols, mainly found in the leaves. Burahol leaves contain several active substances, such as saponins, flavonoids, polyphenols, and isoflavones [8]. Allopurinol is known to be able to reduce uric acid levels through the mechanism of inhibiting the xanthine oxidase enzyme (XO). Common flavonoid compounds in inhibiting xanthine oxidase are apigenin, luteolin, camphorol, quercetin and myresetin. Flavonoids work by reducing the activity of the xanthine oxidase enzyme which is an important enzyme in the process of converting purines to uric acid. It is suspected that the inhibitory activity of the ethanol extract of burahol leaves is supported by flavonoid compounds. S. burahol was found to contain flavonoid compounds from phytochemical screening results. The total flavonoid compounds contained in the ethanol extract were found to be 6.84%. Flavonoids have the ability to form hydrogen bonds, so they can compete with substrates, such as hypoxanthine and xanthine to bind to the xanthine oxidase enzyme, so the ability of the xanthine oxidase enzyme to attach to the substrate will decrease more and more as the amount of flavonoids increases. Other studies also show that some substances in garlic, such as S-allyl-L-cysteine can inhibit enzymes involved in the formation of uric acid, such as XOD, so it may be able to reduce the amount of urate in quail.

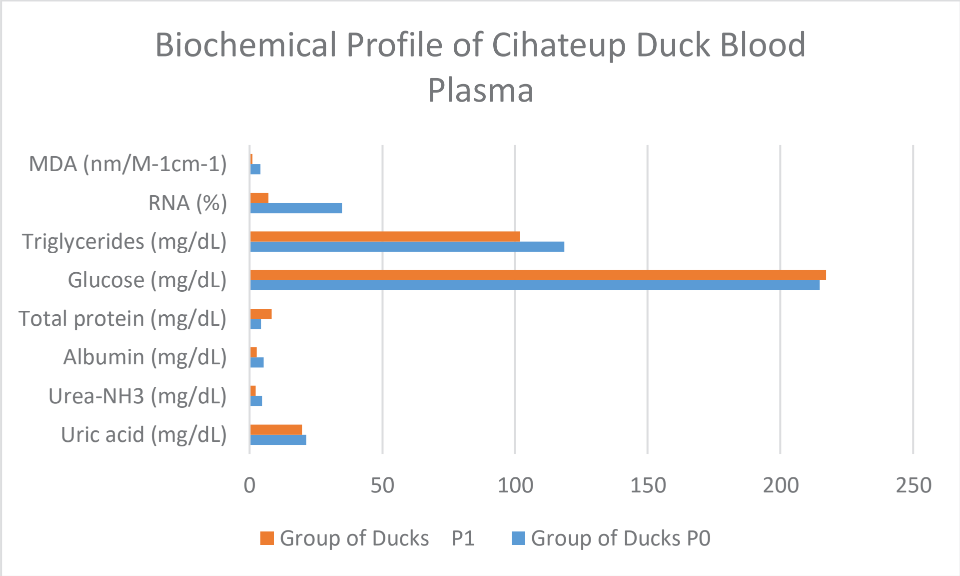

Fig. 1. Biochemical Profile of Cihateup Duck Blood Plasmma

The average urea-NH_3 profile decreased before and after administration of burahol fruit flour, from 4.71 mg/dL to 2.19 mg/dL. Reducing ammonia emissions can be done by stopping the formation of uric acid, helping laying hens digest and absorb protein and amino acids better, slowing down the growth of microorganisms that produce ammonia, and decreasing the activities of enzymes that break down urea and uric acid. [9]. Flavonoids act as natural antioxidants and antimicrobials to inhibit the growth of protein-degrading microbes that cause ammonia formation in the digestive tract. The process of protein decomposition into ammonia can be reduced by reducing microbial activity, resulting in lower ammonia levels in feces and blood. Methionine is an essential amino acid and contains sulfur to cleanse toxins and help make proteins in the body. If the supply of methionine is sufficient, the body can use nitrogen better, urea levels in the blood will decrease, and less nitrogen is excreted as ammonia. Linoleic acid is a type of essential fatty acid and is needed by the body to maintain the condition of cell membranes and liver function. The function of enzymes in the liver in the urea cycle is improved when linoleic acid is combined with flavonoids, so that nitrogen can be used efficiently to build body tissues, rather than being released as urea or ammonia gas.

The average albumin profile decreased before and after administration of burahol fruit flour, from 5.26 mg/dL to 2.75 mg/dL. Albumin is one of the main proteins in the blood that plays a role in maintaining blood pressure, transporting nutrients, and indicating the condition of protein metabolism in livestock. The content of bioactive compounds in burahol fruit flour, such as flavonoids, tannins, and phenols act as antioxidants and liver protectors. This can improve liver function, which is the main organ producing albumin. In addition, the flavonoid content in burahol can also help reduce free radicals and oxidative stress, as the cause of decreased albumin levels in livestock with fast metabolism.

The average total protein profile increased before and after administration of burahol fruit flour, from 4.34 mg/dL to 8.23 mg/dL. Bioactive compounds, such as flavonoids, tannins, and phenols, can improve digestive health by inhibiting the growth of pathogenic bacteria, thus improving nutrient absorption, particularly protein. Furthermore, phenolic compounds in burahol flour help reduce oxidative stress in protein metabolism. Flavonoids help maintain cell membrane stability and protect plasma proteins from free radical damage. Meanwhile, methionine is an essential amino acid that plays a role in the formation of body proteins and enzymes, so increasing the availability of methionine in feed is expected to increase blood protein levels in ducks. Linoleic acid is a polyunsaturated fatty acid involved in cell membrane formation, fat transport, and the formation of prostaglandin hormones that influence protein metabolism and liver function. The results showed that administration of burahol flour mixed with flavonoids, methionine, and linoleic acid significantly increased total blood protein levels in Cihateup ducks compared to untreated ducks. This increase demonstrates that the combination supports optimal protein metabolism and liver function in ducks. Physiologically, total blood protein levels reflect the balance between protein production and breakdown in the body. Higher protein levels indicate an increased ability of the body to produce plasma proteins such as albumin and globulin, which are linked to livestock health and immunity.

The average glucose profile increased after administration of burahol fruit flour, from 214.89 mg/dL to 217.18 mg/dL. Blood glucose levels are maintained stable in the body through the process of homeostasis. This process involves several sources of glucose in the body, such as glycogen, fatty acids, and amino acids. If glucose levels are low, it means the livestock is not getting enough feed. Blood glucose is obtained from food, especially from carbohydrates, as well as other food sources, such as protein and fat. Related to water shortages, the hypothalamus will immediately receive a signal to stimulate the sympathetic nerve. The sympathetic nerve will release the hormone epennephrine which causes blood flow to the surface of the body to increase.

The average triglyceride profile decreased after administration of burahol fruit flour, from 118.64 mg/dL to 101.89 mg/dL. Flavonoids are bioactive substances with strong antioxidant properties. These compounds can neutralize free radicals and reduce the process of fat oxidation in the body. Therefore, flavonoids are able to protect cell membranes from damage due to oxidation and inhibit the fat formation pathway. This occurs because flavonoids can slow the activity of key enzymes such as HMG-CoA reductase and transcription factors. The most effective dose for optimal triglyceride reduction is around 0.04% in the feed. Methionine acts as a source of methyl through the compound S-adenosyl methionine (SAM) and is also a raw material for glutathione, an important antioxidant that works in the body. Thus, methionine supports liver function, the formation of fat proteins, and the regulation of genes related to the process of fat transport. Linoleic acid is an important fatty acid and is a raw material for the synthesis of polyunsaturated fatty acids (PUFA). This acid plays a role in maintaining cell membrane structure and influencing the oxidation reaction pathway and fatty acid combustion. Increasing linoleic acid intake can enhance fat burning, thereby reducing triglyceride accumulation in tissues. The combination of flavonoids, methionine, and linoleic acid works effectively through antioxidant mechanisms, regulating fat metabolism, and enhancing fatty acid combustion, ultimately resulting in lower triglyceride levels in the body. The recommended dosage is around 0.04 to 1.5% of the total diet for maximum and safe effects.

The average reticulocyte RNA profile decreased before and after administration of burahol fruit flour, from 34.83% to 7.05%. Reticulocyte RNA is a sign of the process of protein formation and red blood cell growth (*erythropoiesis*). The combination of flavonoids, methionine, and linoleic fatty acids in burahol fruit flour improves cell metabolism, liver function, and bone marrow that produces reticulocytes. Flavonoids act as powerful antioxidants that reduce oxidative stress in bone marrow and blood-forming tissues. The cellular environment becomes more favorable for the process of RNA and protein synthesis by reducing lipid peroxidation, thereby improving reticulocyte development. Methionine is an important amino acid that supplies methyl through S-adenosylmethionine (SAM), which is involved in the process of DNA and RNA methylation. In addition, methionine is also a raw material for glutathione formation to maintain red blood cell quality and increase the transcription of genes related to erythropoiesis. Reticulocyte RNA levels can decrease if methionine is lacking due to inhibition of erythroid protein synthesis. Linoleic acid (ω-6 PUFA) plays a role in cell membrane structure and cell signaling regulation. Linoleate contributes to the flexibility of cell membranes and the activation of enzymes involved in red blood cell differentiation. However, excessive linoleate can increase fat oxidation, thereby disrupting the stability of reticulocyte RNA. This combination can enhance reticulocyte RNA synthesis, accelerate red blood cell development, and improve blood quality in Cihateup ducks.

The average MDA profile decreased before and after administration of burahol fruit flour, from 4.18 nm/M-1cm-1 to 1.06 nm/M-1cm-1. The main parameter used to measure the level of oxidative stress in livestock is the concentration of malondialdehyde (MDA), which is the end result of the lipid peroxidation process. The higher the MDA concentration, the greater the oxidative damage that occurs to cell membranes, especially to unsaturated fatty acids found in lipoproteins and hepatocyte membranes. Excessive environmental temperatures, high humidity, and limited water access will cause ducks to be uncomfortable and experience increased oxidative stress. This has an impact on the occurrence of free radical attacks on cell membranes. These free radicals cause disruptions in metabolic processes and reduce cell function, such as DNA mutations, failed transcription processes, errors in the translation process, and decreased protein function that results in mutations, toxic effects on cells, and changes in enzyme activity. This has an impact on disruptions to and decreases in overall metabolism. Other studies have shown that the production of free radicals (Reactive Oxygen Species = ROS) increases with increasing environmental temperature. This situation becomes more detrimental to livestock if accompanied by increased air humidity in the barn. Lipid peroxidation is a complex chemical process that occurs when polyunsaturated fatty acids (PUFA) in cell membranes react with ROS, which are highly reactive oxygen compounds. This process produces hydroperoxides, one of which is MDA [10]. ROS include not only free radicals such as hydroxyl radicals (˙HO), superoxide radicals (O_2^-), and nitric oxide (NO), but also other reactive molecules that share an electron pair, such as hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), and peroxynitrite anions. Essentially, ROS attack PUFA in membrane lipids, causing degradation and the formation of MDA. MDA levels in the blood are often used as a marker of cellular damage due to reactions with free radicals. Flavonoids act as free radical scavengers and can reduce lipid oxidation. This compound can also inhibit the activity of peroxidation enzymes, such as HMG-CoA reductase and SREBP1, thereby reducing the formation of excess triglycerides that have the potential to be oxidized. Methionine acts as a methyl group donor (SAM) and is a raw material for the formation of glutathione (GSH), an important antioxidant produced by the liver. GSH can increase the body's ability to neutralize free radicals and repair damage caused by oxidation. Linoleic acid plays a role in the formation of phospholipids that form more stable cell membranes. Linoleate is susceptible to oxidation, and the presence of flavonoids and GSH from methionine can reduce lipid peroxidation reactions. Previous research has shown that administering natural antioxidant supplements from plants can reduce MDA levels in the plasma and liver of poultry. Therefore, administering burahol fruit flour containing flavonoids, methionine, and linoleate is expected to reduce MDA levels in the blood and liver tissue of Cihateup ducks. This indicates a decrease in oxidative stress and an improvement in the quality of lipid metabolism.

2.2 Effect fruit flour (*Stelechocarpus burahol*) in feed on Performance of Cihateup Ducks

Table 2. Performance of Cihateup Ducks

Response	Group of Ducks	
	P0	P1
Average HDP (%)	68.83^{a}	88.97^{b}
Average egg weight (g)	73.73^{a}	84.92^{b}
Shell thickness (mm)	2.01^{a}	3.15^{b}
Average hatching power (%)	65.65^{a}	88.66^{b}

The average followed by a superscript of different alphabetic elements shows a significant difference ($P<0.05$)
Information :
P0: Without giving feed additives
P1: Addition of feed additive for burahol flour *(Stelechocarpus burahol)*

The average HDP profile increased before and after administration of burahol fruit flour, from 68.83% to 88.97%. Flavonoids can reduce inflammation by working in several ways, such as stopping certain enzymes and proteins that help control inflammation-related messengers [11]. They are also strong antioxidants that can neutralize harmful free radicals and reduce how much of them are produced [12]. Flavonoids help the body utilize dietary nutrients better and make digestive enzymes work more efficiently, which can lead to higher egg production. In older hens, flavonoids from burahol leaves increased the activity of T-SOD and lowered MDA levels in the shell gland. This shows that adding burahol-leaf flavonoids improved the shell gland's ability to remove harmful free radicals in aged hens [13]. Flavonoids help reduce oxidative stress by eliminating free radicals that can damage ovarian cells and liver tissue. The process of forming reproductive hormones such as estradiol and progesterone runs better by reducing lipid oxidation and increasing cell membrane stability. These hormones play a role in the ovulation process and egg formation, thus directly increasing daily egg production (HDP). Furthermore, methionine plays a role in protein formation and the formation of egg yolk lipoproteins (vitellogenin and very low density lipoprotein/VLDL) in the liver. Adequate methionine facilitates lipid transport from the liver to the ovaries, accelerates egg yolk formation, and increases ovulation frequency. Methionine also aids the liver's detoxification process and maintains lipid metabolism balance for the physical condition of laying ducks. Linoleate, a polyunsaturated fatty acid (PUFA), plays a role in prostaglandin formation and the structure of reproductive cell membranes. Increasing linoleic acid consumption can improve follicle quality and stimulate ovulation. Linoleic acid also regulates the efficiency of energy use and improves the lipid composition of egg yolk, which supports the quantity and quality of eggs produced. The results of this study are consistent with research which states that flavonoids in the form of isoflavones act as phytoestrogens that encourage vitellogenin formation in the liver. Vitellogenin then enters the bloodstream and travels to the ovaries as one of the raw materials for egg yolk formation. Phytoestrogens also stimulate follicle growth in the ovaries, increasing the number of developing follicles, and consequently, distributing the fat and cholesterol that make up egg yolk to more follicles.

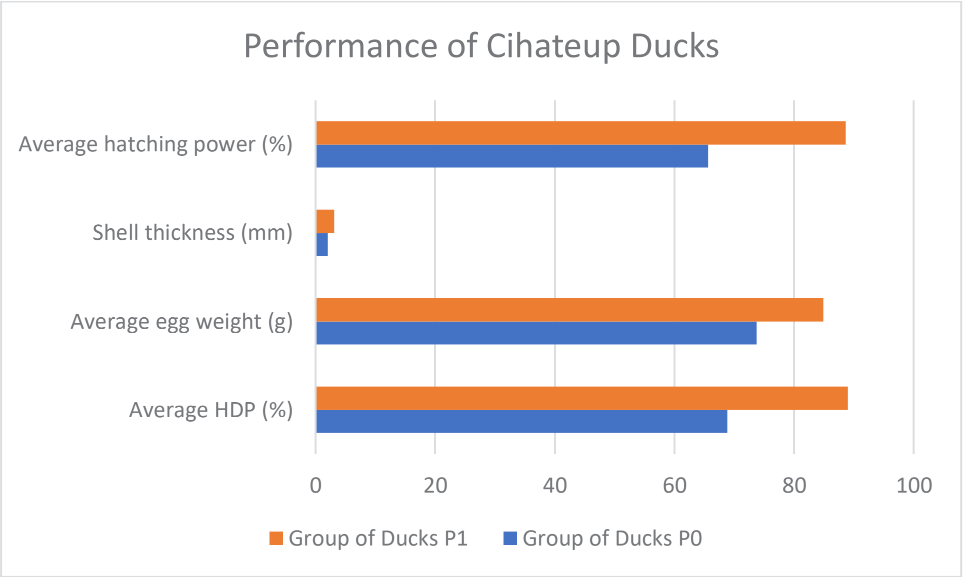

Fig. 2. Performance of Cihateup Duck

The average egg weight profile increased before and after the administration of burahol fruit flour, namely from 73.73 grams to 88.97 grams. Egg weight indicates the total weight of the egg parts, namely the shell and the contents of the egg consisting of the yolk and egg white. The formation of these three parts is influenced by the nutrients contained in the feed, such as protein, fat, carbohydrates, calcium, and phosphorus. The availability of protein and fat in the feed affects the process of duck egg formation, especially at the vitellogenesis stage. Vitellogenesis is the stage of vitellogenin formation which is the raw material for the formation of egg yolk. The increase in the amount of vitellogenin stored in the developing yolk affects egg weight. Protein absorbed through the small intestine is used for the formation of the egg shell, albumin, and vitellogenin. Furthermore, [explained that egg weight reflects the efficiency of protein and energy use in feed. In addition, Methionine supports protein synthesis and lipoprotein formation in the liver for lipid transport to the ovaries in livestock metabolism. The increase in lipoprotein availability helps the formation of egg yolk, so that egg weight can increase significantly. Furthermore, methionine is also a precursor to the endogenous antioxidant glutathione, which helps maintain oxidative balance in reproductive cells. Linoleate plays a role in cell membrane formation and is a major component of triglycerides in egg yolk. The availability of linoleic acid in the feed encourages increased egg fat deposition, improving yolk size and weight without compromising albumen quality. The combination of linoleate, methionine, and flavonoids creates a synergistic effect: flavonoids maintain lipid stability from oxidation, methionine facilitates fat transport and metabolism, and linoleate provides a fat substrate for egg formation.

The average profile of shell thickness increased before and after administration of burahol fruit flour, namely from 2.01 mm to 3.15 mm. Leaf flavonoids are the main useful parts in burahol leaves, and they include many active ingredients such as routine, isoquercitrin, kaempferol 3-(6-rhamnosylglucoside), and quercetin 3-(6-malonylglucoside). These compounds help with health by acting as antioxidants, supporting the immune system, and reducing pain. A study showed that eating burahol leaves can lower liver stress in obese mice caused by a high-fat diet. In recent years, research has shown that flavonoid extracts from plants can act like estrogen, which helps improve the reproductive health of laying hens. Genistein is used as a feed supplement to help hens better use calcium and strengthen their eggshells during the later stages of laying. Quercetin, one of the main flavonoids in mulberry leaves, also acts like a plant-based estrogen. Flavonoids help protect cells in the fallopian tubes, especially those in the uterus (shell gland), from oxidative stress that can disrupt the formation of calcium carbonate ($CaCO_3$) [14]. With better body condition, calcium absorption and mineral deposition in the eggshell become more effective, resulting in thicker and stronger shells. In addition, methionine is an essential amino acid and plays a role as a methyl group donor in the formation of structural proteins such as the shell matrix (ovocleidin and ovocalyxin). This matrix is the basic framework where calcium carbonate is deposited during shell formation. Adequate methionine availability increases protein production in the uterus, strengthens mineral bonds, and makes the shell thicker. Linoleic acid, an essential omega-6 fatty acid, helps maintain healthy cell membranes in the fallopian tubes and supports the metabolism of steroid hormones, especially estrogen, which plays a role in regulating calcium deposition. Linoleate also affects the absorption of vitamin D_3 which is very important for the balance of calcium and phosphorus in the body.

The average hatchability percentage profile increased before and after administration of burahol fruit flour, from 65.65% to 88.66% [15]. Burahol extract acts as an absorbent and prebiotic, thus helping improve digestion and reduce oxidative stress. Flavonoids play a role in reducing free radicals (ROS) that can damage ovarian follicle cells, egg vitelline membranes, and embryonic tissue. Methionine is an essential amino acid for protein formation and functions as a methyl donor through the compound S-adenosylmethionine (SAM). In addition, methionine is also a raw material for the production of glutathione (GSH), an important antioxidant produced by the cells themselves. Providing additional methionine to female poultry can improve egg quality and the likelihood of embryo survival. This is due to an increased oxidation-reduction balance and increased gene expression that helps fight oxidative damage. Linoleate helps form flexible embryonic cell membranes that can penetrate substances. This substance also increases HDL levels and reduces LDL oxidation in the mother's blood. Linoleate ensures an adequate supply of fat energy for the embryo during incubation. According to [15], increasing unsaturated fatty acids in hen feed improves the quality of the yolk membrane and facilitates the transfer of fat to the embryo. Thus, embryo growth becomes more stable, and the risk of hatching failure due to damage to the embryonic membrane can be reduced.

2.3 Equation

1. Arithmetic mean

$$\overline{X} = \frac{\sum X_i}{n}$$

2. Standard Deviation

$$S = \sqrt{\frac{\sum X^2 - \frac{1}{n}\left(\sum X\right)^2}{n-1}}$$

3. Coefficient of Variation (CV)

$$KV = \frac{S}{\overline{X}} \times 100\,\%$$

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4. Calculate the variance of each variable

$$Sx^2 = \frac{\sum xi^2 - \frac{\left(\sum x\right)^2}{n}}{n-1} \qquad Sy^2 = \frac{\sum yi^2 - \frac{\left(\sum y\right)^2}{n}}{n-1}$$

Description:
Sx2 = Laying hen variety with feed additives (FA)
Sy2 = Laying hen variety without FA

Calculating uniformity

$F = \frac{Large\ variance}{Small\ Variance}$, $\alpha\ (n_1\text{-}1;\ n_2\text{-}1)$

If: F count ≤ Fα = Equal variance
F count > Fα = Unequal variance

Note:
F = Population uniformity
n1 = Number of laying hens with FA given
n2 = Number of laying hens without FA given
For equal variance

$$Sa = \sqrt{Sp2\ (\frac{1}{n1} + \frac{1}{n2})}$$

Where

$$Sp^2 = \frac{(n1-1)Sx + (n2-1)\ Sy2}{n1+n2-2}$$

Description:
Sa = Variance
Sp^2 = Combined variance of laying hens with and without FA treatment
Sx^2 = Variance of laying hen samples with FA treatment
Sy^2 = Variance of laying hen samples without FA treatment
Sx^2 = Average parameter of laying hen samples with FA treatment
$\bar{x}$ = Average parameter of laying hen samples without FA treatment
$\bar{y}$ = Average parameter of laying hen samples with FA treatment

5. For unequal variances

$$\bar{d} = \sqrt{\left(\frac{sx^2}{n_1} + \frac{sy^2}{n_2}\right)}$$

Hypothesis
H0 = There is no difference in neutrophil and lymphocyte levels in chickens fed with or without FA.

H1 = There is a difference in neutrophil and lymphocyte levels in chickens fed with or without FA.
If the calculated t-value is less than the table t-value, H0 is accepted.
If the calculated t-value is greater than the table t-value, H0 is rejected.

Test Statistic : $t = \frac{\bar{x}-\bar{y}}{Sd}$

The calculated t value obtained is compared with the value

$$t'\alpha = \frac{t\alpha\ ; n_1 - 1\ W_1 + t\alpha\ ; n_2 - 1\ W_2}{w_1 + w_2}$$

$$w_1 = \frac{s_x{}^2}{n_1} \qquad dan \qquad w_2 = \frac{s_y{}^2}{n_2}$$

Description:
t' = t' calculated for comparison α
tα; n1-1 = t-table value for row α and sample column n1-1
tα; n2-1 = t-table value for row α and sample column n2-1
W1 = Ratio of deviation/variance of laying hens fed FA to the total sample
W2 = Ratio of deviation/variance of laying hens not fed FA to the total sample

3 Conclusion

The administration of Burahol fruit flour 15 g/kg ration had a significant effect (P<0.05) on the biochemical profile of blood plasma of cihateup ducks, namely uric acid (19.67 mg/dL), urea (NH_3) (2.19 mg/dL), albumin (2.75 mg/dL), total protein (8.23 mg/dL), glucose (217.18 mg/dL), triglycerides (101.89 mg/dL), RNA (7.05%), and MDA (1.06 nm/M-1cm-1), as well as the performance of cihateup ducks, namely an average egg production of 88.97%, egg weight of 84.9 grams, shell thickness of 3.15 m, and an average hatchability of 88.66%.

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