



Effect of the Addition Red Beetroot Powder (*Beta vulgaris L. Var. Rubra L*) as a Feed Additive in Feed on Production Performance, Egg Yolk Cholesterol and Blood Profile of Laying Hens

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ABSTRACT: The aim of this research was to evaluate the effect of addition red beetroot powder (*Beta vulgaris L. Var. Rubra L*) as a feed additive in feed on production performance, egg yolk cholesterol and blood profile of laying hens. The first stage of research included testing the inhibitory power of *Escherichia coli*, *Salmonella sp*, and Lactic Acid Bacteria (LAB). The materials used in the bacterial inhibition test were red beetroot pulp powder, red beetroot peel powder and red beetroot pulp and peel powder. The method used was *in vitro research* and the data was analyzed statistically using the ANOVA test. If there were differences between each treatment, it was continued with Duncan's Multiple Range Test. The second stage of research is the field application of the results of the first stage research *in vivo*. The material used was 120 laying hens of the *isa brown strain* aged 30 weeks. The method used was an experimental method using a Completely Randomized Design (CRD) with 4 treatments and 6 replications, each using 5 chickens. Data were analyzed statistically using the ANOVA test. If there were differences between each treatment, it was continued with Duncan's Multiple Range Test. Variables observed in the second stage of research included production performance, egg yolk cholesterol and blood profile of laying hens. The results of the first stage of research showed that the use of red beetroot plant powder had a very significant effect ($T < 0.01$) on the diameter of the inhibition zone for *Escherichia coli*, *Salmonella sp* and Lactic Acid Bacteria (LAB). Red Beetroot Pulp and Peel Powder (RBPPP) has the best antibacterial power so it was applied in the second stage of research. The results of the second stage of research showed that the addition of RBPPP had a significant effect ($T < 0.05$) on feed consumption, had a very real effect ($T < 0.01$) on egg weight, HDP, egg mass, feed conversion, IOFC, hemoglobin, erythrocytes and hematocrit. However, there was no significant effect ($T > 0.05$) on egg yolk cholesterol and leukocytes. The addition of RBPPP at the 1% level has the potential to reduce feed consumption and feed conversion ratio, increase egg weight, HDP and egg mass, even though the IOFC value is lower than the control treatment or without RBPPP. The addition of RBPPP at a level of 1.5 % has the potential to reduce egg yolk cholesterol levels compared to other treatments. The addition of RBPPP up to a level of 1 % is able to increase and maintain hemoglobin and hematocrit levels in normal conditions. Apart from that, the addition of RBPPP at levels of 0.5 % and 1.5 % is able to increase and maintain erythrocyte and leukocyte levels in normal conditions.

KEYWORDS: Red beetroot pulp and peel powder, feed additive, production performance, egg yolk cholesterol, blood profile, laying hens

INTRODUCTION

Indonesia is a country with a very fast population increase, causing food needs to also increase. One food source that is very important for human growth and health is protein (Santoso, 2022). The source of protein can be animal protein from livestock. One of the livestock commodities that is increasing is laying hens. Laying chickens are one of the superior poultry commodities which function as egg producers to support the availability of protein that is easy to obtain and cheap for various levels of society (Herianto, Fenita, Santoso, Brata and Suharyanto, 2022). Based on data from the Central Statistics Agency (2022), chicken egg production in Indonesia throughout 2022 will reach 5,600,000 tons. This production volume increased by 7.69 % compared to 2021, namely 5,200,000 tons. Haryuni, Widodo and Sudjarwo (2017) said that the keys to success in increasing production include breeding, feeding and management.

Feed is an important factor in the laying hen farming business that requires more attention. This is because feed has a very important role both from an economic and production perspective. Hasjidla, Cholissodin and Widodo (2018) stated that feed costs



account for 60-70% of the total production costs in laying chicken farming businesses. Production costs on a farm can be minimized if the efficiency of the feed used to produce eggs increases. High feed efficiency will be achieved if the chicken's digestive tract is in optimal conditions for digesting and absorbing food substances. Efforts that can be made to increase feed efficiency are by adding *feed additives* to the feed. *Feed additives* are materials that do not include food substances that are added to feed in small quantities with the aim of stimulating growth and increasing the population of beneficial microbes in the digestive tract. *Feed additives* function as growth triggers and increase feed efficiency in chickens (Nuningtyas, 2014). *Feed additives* are grouped into several types, namely antibiotics, organic acids, enzymes, phytobiotics, prebiotics, probiotics and synbiotics. Antibiotics have been widely applied by laying hen breeders to increase the efficiency of feed use and increase livestock productivity, however the use of antibiotics as *feed additives* was banned by the Indonesian government in January 2018. The regulation prohibiting the use of antibiotics as *feed additives* is contained in article 16 of Minister of Agriculture Regulation No. 14/ 2017 concerning classification of veterinary drugs (Hidayat and Rahman, 2019).

One alternative that can be done to reduce the use of antibiotics is to use *feed additives* in the form of phytobiotics, because antibiotics have an adverse risk for human health because antibiotic residues in livestock products can cause resistance in the bodies of livestock which are then consumed by humans. According to Ramiah, Zulkifli, Rahim, Ebrahimi, and Meng (2014) phytobiotics are herbal plants that have antibacterial active ingredients so they can improve the condition of the digestive tract, balance pH and the number of microflora, feed conversion ratio and improve production performance. Giving phytobiotics to poultry can increase growth and feed efficiency, improve intestinal histomorphology and function as an antimicrobial so that it can increase the immune system of poultry (Hosseini, Lamers, Soltani, Meijer, Spaik, and Scaaf, 2016).

One herbal plant that can be used as a phytobiotic is red beetroot. Beetroot (*Beta vulgaris L*) are plants belonging to the *Chenopodiaceae* family and have many varieties ranging from white to red. The dark red variety is very popular because it is widely consumed by the public (Singh and Hathan, 2014). Beetroot are grown in many parts of the world, especially in Asia, North Africa, Europe and North America (Kowalski and Szadzinska, 2014). In recent years, red beetroot have become increasingly popular in physiological and biological activities due to their effect as a functional food in terms of health (Clifford, Howatson, West and Stevenson, 2015). Red beetroot is rich in valuable active compounds such as carotenoids, betalains, polyphenols, flavonoids and saponins (Kaur and Singh, 2014). Red beetroot contain many color pigments (betalains), contain substances such as the antioxidants betanin, isobetanin, vulgaxanthin and various compounds with antimicrobial properties. Red beets are also rich in vitamins. This plant is very rich in vitamins A, B and C, and contains Fe, Mg, Ca, K and folic acid. As a powerful antioxidant, beets support blood production in the body, increase the number of red blood cells and strengthen the immune system. Another important effect of red beetroot is to strengthen the stomach and intestinal system, facilitate digestion and regulate intestinal activity (Sengul, 2021). Waiz (2023) reported that the addition of 25 ml of beetroot juice per liter of drinking water was able to improve growth performance, PCV and Hb and reduce glucose, cholesterol and triglyceride levels in kadaknath chickens. Apart from that, giving beetroot powder at a level of 15-30 gram/kg feed can significantly increase production performance in geese (Al-waeli, Alasadi and Abbas, 2021). The addition red beetroot powder to quail feed up to 1 % has a positive impact on egg yield, egg yolk color, and palmitic and linolenic acid values (Sengul, 2021).

The use of beetroot plants in research in the livestock sector is still rarely carried out. The limited research information that uses beetroot as a *feed additive* in poultry feed is the reason for the need to carry out further research and as an answer to follow up on regulations related to prohibiting the use of antibiotics. Based on the description above, we will study further the addition red beetroot powder (*Beta vulgaris L. Var. Rubra L*) as a *feed additive* in feed on production performance, egg yolk cholesterol and blood profile of laying hens.

MATERIALS AND METHODS

The first stage of research was carried out at the Plant Pest and Disease Laboratory (PPD) of the Faculty of Agriculture, Brawijaya University on January 10 2024 . The research materials in the first stage were Red Beetroot Pulp Powder (RBPP), Red Beetroot Peel Powder (RBPP) and Red Beetroot Pulp and Peel Powder (RBPPP), bacterial isolates using *Escherichia coli*, *Salmonella sp* . and Lactic Acid Bacteria (LAB). This research uses the *in vitro* well diffusion method. The design used was a Completely Randomized Design (CRD) using 4 treatments and 2 replications . The treatment is as follows:

T0 = Bacterial isolate + aquades



T1 = Bacterial isolate + concentration solution of RBPP 75%

T2 = Bacterial isolate + concentration solution of RBPP 75%

T3 = Bacterial isolate + concentration solution of RBPPP 75%

The variable observed was the diameter of the inhibition zone for *Escherichia coli* bacteria, *Salmonella sp.* and Lactic Acid Bacteria (LAB). Data were analyzed using analysis of variance or ANOVA test, if there were differences between each treatment then Duncan's Multiple Range Test was continued.

The best research results from the first stage were continued in the second stage of research. The second stage of research consisting of raising laying hens was carried out at Tandjaja Farm which is located in Lamong Village, Badas District, Kediri Regency, a proximate analysis was carried out in the Animal Nutrition and Food Laboratory, Faculty of Animal Husbandry, Brawijaya University, an analysis of egg cholesterol observations was carried out in the Analysis Laboratory and Measurements from the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Brawijaya University and a blood profile analysis carried out at the Indonesian Healthy Animal Laboratory, Malang. The second stage of research material used 120 laying hens of the *isa brown strain* aged 30 weeks with a coefficient of diversity of 2.65%. The feed used is *semi-self mixing* feed with a protein content of 19.82%. The method used was an *in vivo experimental method* with a Completely Randomized Design (CRD) consisting of 4 treatments and 6 replications. Each replication unit consisted of 5 laying hens as experimental units. The treatment is as follows:

T0 = Basal feed without the addition of RBPPP

T1 = Basal feed + RBPPP 0.5 %

T2 = Basal feed + RBPPP 1 %

T3 = Basal feed + RBPPP 1.5 %

The variables observed were feed consumption, egg weight, HDP, *egg mass*, FCR, IOFC, egg yolk cholesterol, hemoglobin, erythrocytes, hematocrit and leukocytes. Data were analyzed using analysis of variance or ANOVA test, if there were differences between each treatment then Duncan's Multiple Range Test was continued.

RESULTS AND DISCUSSION

Research on the addition of red beetroot powder (*Beta vulgaris L. Var. Rubra L*) was divided into two research stages. In the first stage, there was a test of the inhibitory power of Red Beetroot Pulp Powder (RBPP), Red Beetroot Peel Powder (RBPP) and Red Beetroot Pulp and Peel Powder (RBPPP) on Lactic Acid Bacteria (LAB), *Escherichia coli* and *Salmonella sp.* *in vitro*. The best results from the first stage of research were applied to the second stage of research *in vivo* to determine response the addition red beetroot powder as a *feed additive* in feed on production performance, egg yolk cholesterol and blood profile of laying hens. The complete results of the two research stages are as follows:

Phase I Research: Bacterial Inhibition Testing

The bacterial inhibition test aims to measure how much the ability or concentration of a compound can have an effect on microorganisms. The inhibitory power of bacterial growth is caused by the relationship between active compounds through attachment or diffusion of antimicrobial substances with bacteria. Research on the inhibitory power of *Escherichia coli*, *Salmonella sp.* and Lactic Acid Bacteria (LAB) aims to determine differences in the bacterial inhibitory power of the parts of the red beet plant that provide the best results, continuing in phase II research. Data from research at the preliminary stage, namely the effect of the red beetroot plant powder on bacterial inhibition, is shown in Table 1.

Table 1. Effect of Treatment on Bacterial Inhibition Test

Treatment	Bacterial Inhibition Zone Diameter (mm)		
	<i>Escherichia coli</i>	<i>Salmonella sp.</i>	Lactic Acid Bacteria (LAB)
T0	0±0 ^a	0±0 ^a	0±0 ^a
T1	2.15±0.07 ^b	2.60±0.28 ^{ab}	1.20±0.14 ^b
T2	5.30±0.28 ^c	5.30±0.14 ^{bc}	4.30±0.14 ^c
T3	9.55±0.49 ^d	11.15±1.34 ^d	8.60±0.28 ^d

Note: Different notations in the same column indicate very significant differences ($T < 0.01$) between treatment.



Effect of Treatment on Pathogenic Bacteria (*Escherichia coli* and *Salmonella sp.*)

The results of analysis of variance showed that different parts of the beet plant gave very significant differences ($T < 0.01$) to the bacterial inhibition test of *Escherichia coli* and *Salmonella sp.* The research results in Table 1 show that treatment T1 (RBPP) is the part of the beet plant that has the lowest inhibitory ability as indicated by the small average diameter of the inhibition zone from all tests and treatments, namely (2.15 ± 0.07) mm for *Escherichia inhibition. coli* and (2.60 ± 0.28) mm on the inhibition of *Salmonella sp.* This was followed by treatment T2 (RBPP) which had an inhibition zone diameter of (5.30 ± 0.28) mm for inhibiting *Escherichia coli* and (5.30 ± 0.14) mm for inhibiting *Salmonella sp.* Treatment T3 (RBPPP) is the part of the beet plant that has the highest inhibitory ability among all treatments when compared to treatments T1 and T2, namely (9.55 ± 0.49) mm for *Escherichia coli* and (11.15 ± 1.34) mm for *Salmonella sp.* This result is relatively lower when compared to research reported by Salamatullah, Hayat, Alkaltham, Ahmed, Arzoo, Husain, Al-Dossari, Shamlan and Al-Harbi (2021) that beetroot flesh has an inhibition zone of 21 mm and beet skin has a 17 mm barrier against *Escherichia coli* bacteria.

The traditional use of beetroot for the treatment of infectious diseases provides the basis for its inhibitory effect against pathogenic microorganisms (Kumar and Brooks, 2017). Beetroot (*Beta vulgaris*) which are included in the *chenopodiaceae* are rich in betalain compounds which are the plant with the 10th highest antioxidant content (Kumar and Brooks, 2017). Betalain pigment is the main antioxidant which can protect red beets from wound damage or the presence of bacteria so that it can function as an antimicrobial (Amila, Siti, Henny, Jon and Vierito, 2021).

The ability of T1 (RBPP) and T2 (RBPP) treatments to inhibit the pathogenic bacteria *Escherichia coli* and *Salmonella sp.* categorized in the weak zone because it has an inhibitory zone diameter ≤ 5 mm. Meanwhile, the T3 (RBPPP) treatment which has the best antibacterial potential compared to other treatments in inhibiting the pathogenic bacteria *Escherichia coli* is categorized in the medium zone because it has an inhibitory zone diameter of 6-10 mm and in inhibiting the pathogenic bacteria *Salmonella sp.* categorized in the strong zone because it has an inhibitory zone diameter of 11-20 mm. The results of this research are in accordance with the statement by Salamatullah, *et al.*, (2021) that the red beetroot pulp and peel powder show the highest total polyphenol, total flavonoid and antibacterial potential content compared to other parts.

Phenolic compounds have an important role as antibacterials by increasing the permeability of the cytoplasmic membrane, resulting in loss of cellular pH gradient, decreased ATP levels and loss of proton motive power which causes cell death in gram-negative and gram-positive bacteria (Canadanovic-Brunet, Savatovic, Cetkovic, Vulic, Djilas and Markov, 2011). The way flavonoids work is to denature bacterial cell proteins, as a result the bacterial cells cannot be repaired (Setyorini, Rahayu and Sistyaningrum, 2017). Nakayama, Shimatani, Ozawa, Shigemune, Tsugukuni, Tomiyama, Kurahachi, Nonaka and Miyamoto (2013) reported that flavonoids will interact with protein porins found on the outer membrane of *Escherichia coli* bacteria, which will then inhibit the function of porins as transporters of small hydrophilic molecules such as glucose. Inhibition of this porin function will further inhibit the growth of *Escherichia coli* bacteria. Xie, Yang, Tang, Chen and Ren (2015) reported that flavonoids can change the level of cell membrane permeability which can reduce pathogenic bacteria. Flavonoids can also change the structure of the outer part and cytoplasmic membrane which results in disruption of the exchange of metabolites and nutrients which then results in the cessation of energy supply for bacteria (Eumkeb and Chukrathok, 2013).

Effect of Treatment on Non-Pathogenic Bacteria (Lactic Acid Bacteria)

The results of the analysis of variance showed that different parts of the beetroot plant provided very significant differences ($T < 0.01$) in the bacterial inhibition test for Lactic Acid Bacteria (LAB). The research results in Table 1 show that treatment T1 (RBPP) is the part of the beet plant that has the lowest inhibitory ability as indicated by the small average diameter of the inhibition zone from all tests and treatments, namely (1.20 ± 0.14) mm, followed by treatment T2 (RBPP) which had an inhibition zone diameter of (4.30 ± 0.14) mm. Treatment T3 (RBPPP) is the part of the beet plant that has the highest inhibitory capacity among all treatments when compared to treatments T1 and T2, namely (8.60 ± 0.28) mm.

The ability of treatments T1 (RBPP) and T2 (RBPP) to inhibit Lactic Acid Bacteria (LAB) is categorized in the weak zone because it has an inhibitory zone diameter of ≤ 5 mm. Meanwhile, treatment T3 (RBPPP) is categorized in the medium zone because it has an inhibitory zone diameter of 6-10 mm. Lactic Acid Bacteria (LAB) are a group of gram-positive bacteria, while *Escherichia coli* and *Salmonella sp.* is a group of gram-negative bacteria. The results showed that gram-positive bacteria had a lower zone of inhibition than gram-negative bacteria in all treatments. This is in accordance with research reported by Salamatullah, *et al.*, (2021) that all beetroot extracts show a higher inhibition zone against gram-negative bacteria than gram-positive bacteria. . This may be

caused by differences in the cell wall structure of gram-negative bacteria and gram-positive bacteria. The cell wall of gram-positive bacteria is made of a thick layer of peptidoglycan with covalently bound teichuronic and teichoic acids making it less susceptible to the action of the test agent.

Phase II Research: Field Application of Phase I Research Results

Phase II research aims to determine and evaluate the effect of the best research results from phase I, namely red beetroot pulp and peel powder in feed as a *feed additive* on production performance, egg yolk cholesterol and blood profile of laying hens.

Effect of Treatment on Production Performance of Laying Hens

Table 2 shows the effect of addition red beetroot pulp and peel powder in feed on the production performance of laying hens. The results showed that the addition of RBPPP had a significant effect ($T < 0.05$) on feed consumption. Apart from that, RBPPP had a very significant influence ($T < 0.01$) on egg weight, HDP, *egg mass*, feed conversion ratio and IOFC.

Table 2. Effect of the Addition RBPPP on Production Performance of Laying Hens

Variable	Treatment			
	T0	T1	T2	T3
Feed Consumption* (g/tail/day)	116.17±0.40 _{abc}	116.52±0.36 ^c	115.83±0.32 ^a	116.08±0.22 ^{ab}
Egg Weight (g/item)**	57.24±0.43 ^a	59.63±1.05b ^c	60.19±0.35 ^c	58.78±0.42 ^b
Hen Day Production (HDP) (%)**	94.65±0.45 ^a	96.79±0.39 ^b	97.68±0.20 ^d	96.85±0.62 ^{BC}
Egg Mass (g/tail/day)**	54.17±0.43 ^a	57.71±1.16 ^{BC}	58.78±0.41 ^c	56.92±0.52 ^b
Feed Conversion Ratio (FCR)**	2.18±0.02 ^d	2.04±0.04 ^b	1.98±0.02 ^a	2.06±0.02 ^{bc}
IOFC (Rp/item)**	683.83±13.20 ^d	618.33±27.67 ^c	504.67±11.41 ^b	312.83±13.47 ^a

Information: * Different notations on the same line indicate significant differences between treatments ($T < 0.05$).

** Different notations on the same line indicate a very significant difference between treatments ($T < 0.01$).

Feed Consumption

Based on the results of the analysis of variance, it showed that the addition of RBPPP to the feed made a significant difference ($T < 0.05$) to the average daily feed consumption of laying hens. The results of the study showed that the feed consumption of laying hens in treatment T2 (with the addition of 1% RBPPP) produced the lowest consumption value compared to other treatments, namely (115.83 ± 0.32) g/head/day, then sequentially the average feed consumption of the highest to lowest values for treatments T1, T0 and T3 were as follows (116.52 ± 0.36), (116.17 ± 0.40) and (116.08 ± 0.22) g/head/day.

Based on DMRT, P2 feed consumption was lower than T1 but not significantly different from T0 and T3. Adding RBPPP to feed at a level of up to 0.5 % can increase feed consumption for laying hens, although at levels of 1 % and 1.5 % giving RBPPP feed consumption tends to decrease. This indicates that feed with the addition of RBPPP at the 0.5 % level has good palatability. This is in line with the opinion of Bozkurt, Kucukyilmaz, Pamukcu, Cabuk, Alcicek and Catli (2012) explaining that phytobiotics can improve the sensory characteristics of feed so that they can increase palatability and stimulate increased feed consumption. In addition, Zhao, Yang, Yang, Wang, Jiang and Zhang (2011) reported that the antioxidant content of phytobiotics can improve the oxidative stability of feed. This will prevent the rancidity process from occurring, so it will also stimulate increased feed consumption.

The research results are higher than the research reported by Souza, Lima, Martins, Assuncao, Junior, Silva and Silva (2019) that the average feed consumption for laying hens with the addition of beetroot powder at a feeding level of 0.8 % was 97.97 grams/tail/day. This increase in feed generally causes an increase in protein and other nutrients consumed by chickens. The large amount of nutrients that enter the chicken's body and are properly absorbed will be used for the needs of life, growth and productivity of the livestock itself. Good feed absorption occurs due to good chicken digestive tract health. This is due to the presence of



phytochemical compounds in the red beetroot pulp and peel powder which act as antioxidants and antibacterials so that they can improve the health of laying hens, especially in the digestive tract.

Beetroot contain several secondary metabolite compounds, namely tannins, saponins, alkaloids, flavonoids, glycosides, steroids and terpenoids (Putra, Safitri, Bisam and Shinta, 2022). The decrease in feed consumption when adding RBPPP by 1 % and 1.5 % is thought to be due to the presence of substances such as saponin which can cause a bitter taste so that chickens will reduce feed consumption due to the presence of RBPPP at higher levels. This is in accordance with Alamgir's (2018) statement that almost all saponins have a bitter and unpleasant taste.

The results of the average daily feed consumption in this study showed that laying hens aged 30-37 weeks during the laying period (*layer*) were able to feed consumption of 115.83-116.52 g/tail/day. The feed consumption achieved in this study was slightly higher than the average standard feed consumption for *isa brown* strain laying hens namely 115 g/tail/day at 30-37 weeks of age (Hendrix Genetic, 2022). Suprijatna and Natawihardja (2014) said that the amount of feed consumed by livestock depends on the quality of the feed ingredients used in preparing the ration, the harmony of the feed composition and nutritional value according to the needs for optimal growth and production. Wulandari, Murningsih and Wahyuni (2012) stated that there are several factors that influence feed consumption, including environmental temperature, livestock condition, metabolic energy balance, crude fiber content of feed, physiological status and age of livestock. The addition of RBPPP to laying hen feed up to a level of 1.5 % does not cause negative effects for laying hens. This confirms that RBPPP is a natural *feed additive* that can be responded well by laying hens.

Egg Weight

Based on the results of the analysis of variance, it showed that the addition of RBPPP to the feed made a very significant difference ($T < 0.01$) to the average egg weight. The results of the research showed that the feed consumption of laying hens in the control or T0 treatment (without the addition of RBPPP) produced the lowest egg weight value compared to other treatments, namely (57.24 ± 0.43) g/item, then sequentially the average feed consumption of the values highest to lowest for treatments T2, T1 and T3 as follows (60.19 ± 0.35) , (59.63 ± 1.05) and (58.78 ± 0.42) g/item.

Based on DMRT, T0 egg weight was lower than other treatments using RBPPP. The addition of RBPPP to feed at a level of up to 1 % can increase egg weight, but there is a decrease in egg weight when the level of RBPPP is increased to 1.5 % but it is still high compared to the control or T0 treatment. The T2 treatment had lower feed consumption than the control or T0 treatment but was able to produce the highest egg weight. This is thought to be because the mineral content in red beetroot pulp and peel powder can be used to increase the activity of enzymes involved in protein synthesis and digestion. Aziz, Dewi and Wirapartha (2020) stated that egg weight is influenced by livestock, calcium and energy content in feed and the environment. Minerals are very important for the functioning of the pancreatic carboxypeptidase A and B enzymes which digest peptides into amino acids, the dipeptidase enzyme which breaks down proteins in digestion so they can be absorbed. This increase in digestibility results in better intake of food substances so that the eggs produced are larger.

The results of the T2 study with the level of red beetroot pulp and peel powder were 1 % higher than the research reported by Souza, et al., (2019) that the average weight of eggs with the addition of beetroot powder with a giving level of 0.8 % was 59.82 g/item. This is because there is an increase in the level of 0.2 %, but when the level is increased to 1.5 % the egg weight decreases. Treatments T1 and T3 had lower results, presumably due to the possibility of excess minerals and active substances in red beetroot bulb and husk powder which are not easily digested by chickens, which produce nutrients including amino acids, protein and methionine, phosphorus and fatty acids. not completely absorbed in the chicken's body.

The results of the average egg weight in this study show that laying hens aged 30-37 weeks of laying period (*layer*) are able to produce eggs of 57.24-60.19 g/item. The egg weight achieved in this study was lower than the average standard feed consumption for *isa brown strain* laying hens namely 61.6-62.9 g/item at 30-37 weeks of age (Hendrix Genetic, 2022). Several factors determine egg weight, one of which is feed consumption and HDP (Setiawati, Afnan and Ulupi, 2016). Apart from that, the nutrients contained in feed such as protein and amino acids also play a role in influencing egg weight.

Hen Day Production (HDP)

Based on the results of the analysis of variance, it shows that the addition of RBPPP in the feed makes a very significant difference ($T < 0.01$) to the average HDP. The results showed that the HDP of laying hens in the control or T0 treatment (without the



addition of RBPPP) produced the lowest egg weight value compared to other treatments, namely $(94.65 \pm 0.45) \%$, then sequentially the average feed consumption was from highest to lowest value for treatment T2, T3 and T1 as follows (97.68 ± 0.20) , (96.85 ± 0.62) and $(96.79 \pm 0.39) \%$.

Based on DMRT HDP P0 is lower than other treatments using RBPPP. The addition of RBPPP to feed at a level of up to 1 % can increase the HDP value, but it decreases when the level of RBPPP is increased to 1.5 % but is still high compared to the control or T0 treatment. Egg production that received RBPPP treatment increased compared to control feed or no treatment. This is thought to be because the feed is optimally absorbed in the intestine due to the addition of red beetrot pulp and peel powder as an antibacterial and antioxidant. Effective nutrient absorption in the digestive tract due to the presence of antibacterials will affect egg production. This is in line with the opinion of Sukrayana, Atmomarsono, Yunianto and Supriyatna (2016) who state that digestibility can be influenced by the level of feeding, type of feed, livestock species, method of processing feed and the health of the digestive tract.

The average HDP results in this study show that laying hens aged 30-37 weeks of laying period (*layer*) are able to produce a daily egg percentage of 94.65-97.68 %. The HDP achieved in this study was almost close to the average standard HDP of laying hens of the *isa brown strain* namely 96.1-96.5 % at 30-37 weeks of age (Hendrix Genetic, 2022). The higher the HDP value, the better the egg productivity. To produce high daily egg production, the ration must provide a balance of protein and energy as well as sufficient vitamin, mineral and water content for basic living needs and the productivity of laying hens. On the other hand, if the nutritional content in the ration is below standard, the chicken's weight will decrease. This will indirectly reduce chickens' daily egg production (Agustina and Purwanti, 2012).

Egg Mass

Based on the results of the analysis of variance, it shows that the addition of RBPPP to the feed makes a very significant difference ($T < 0.01$) to the average *egg mass*. The results showed that *the egg mass* of laying hens in the control or T0 treatment (without the addition of RBPPP) produced the lowest *egg mass value* compared to other treatments, namely (54.17 ± 0.43) g/tail/day, then sequentially the average *egg mass* of The highest and lowest values for treatments T2, T1 and T3 were as follows (58.78 ± 0.41) , (57.71 ± 1.16) and (56.92 ± 0.52) g/tail/day.

Based on DMRT *egg mass* T0 is lower than other treatments using RBPPP. The addition of RBPPP to feed at a level of up to 1 % can increase *egg mass*, but there is a decrease in *egg mass* when the level of RBPPP is increased to 1.5 % but it is still high compared to the control or T0 treatment. The T2 treatment which was able to produce the highest *egg mass* was influenced by the high egg weight and HDP values during the research. This is in line with the opinion of Widjastuti and Kartasudjana (2006) who state that if one or both factors (egg weight and HDP) are higher, the *egg mass* will also increase and vice versa.

The results of the T2 study with the level of red beetrot pulp and peel powder being given were 1 % higher than the research reported by Souza, et al., (2019) that the average *egg mass* with the addition of beetroot powder with a giving level of 0.8 % was 32.81 g/tail/day. This is because there is an increase in the level of 0.2 %, but when the level is increased to 1.5 % the egg mass decreases. The results of the average *egg mass* in this study show that laying hens aged 30-37 weeks of laying period (*layer*) are capable of producing an egg mass of 54.17-58.78 g/tail/day. The egg mass achieved in this study was lower than the average standard feed consumption for *isa brown strain* laying hens namely 59.4-60.5 g/tail/day at 30-37 weeks of age (Hendrix Genetic, 2022). Apart from that, egg mass is also influenced by *feed additives*, feed consumption, egg size and egg weight (Swiatkiewiczet, Wlosek, Krawczyk, Szczurek, Puchala, Jozefakal, 2018).

Feed Conversion Ratio (FCR)

Based on the results of analysis of variance, it shows that the addition of RBPPP to feed makes a very significant difference ($T < 0.01$) to the average feed conversion ratio. The results of the research showed that the feed conversion ratio of laying hens in treatment T2 produced the lowest value compared to other treatments, namely (1.98 ± 0.02) , then sequentially the average feed conversion ratio from highest to lowest value for treatments T0, T3 and T1 was as follows (2.18 ± 0.02) , (2.06 ± 0.02) and (2.04 ± 0.04) .

Based on DMRT, T2 feed conversion with a RBPPP level of 1 % is lower than other treatments or as the best treatment with a value of 1.98, which means that to produce 1 kg of eggs, 1.98 kg of feed is needed. The lower the feed conversion ratio value, the higher the level of feed use efficiency. This is in accordance with the opinion of Luthfi, Nur and Anggraeni (2015) who state that the lower the feed conversion ratio value produced, the better it will be, conversely, if the feed conversion ratio value is high,



the efficiency of feed use will be worse. This situation indicates that feed with the addition of RBPPP can be absorbed well in the chicken's body, especially in the chicken's digestive tract. Apart from that, it is suspected that the flavonoid and antioxidant content contained in RBPPP can maintain the health of chickens, especially the health of the digestive tract of laying hens. The healthy condition of the chicken's digestive tract will support the feed absorption process, thereby having a positive impact on the production performance of laying hens. If the livestock is in healthy condition, the amount of microflora, especially gram-positive microflora, in the digestive tract remains stable (Widodo, 2018).

The results of research with a level of giving red beetroot bulb and husk powder up to a level of 1.5 % were lower than the research reported by Souza, *et al.*, (2019) that the average feed consumption with the addition of beetroot powder with a level of 0.8% was 2.4. The value is higher than the research results so the level of feed use efficiency is not good. The results of the average feed conversion ratio in this study showed that laying hens aged 30-37 weeks during the laying period (*layer*) were able to produce a feed conversion value of 1.98-2.18. The feed conversion ratio achieved in this study was higher than the average standard feed conversion ratio for *isa brown strain* laying hens namely 1.90-1.94 at 30-37 weeks of age (Hendrix Genetic, 2022). The feed conversion ratio value illustrates the good efficiency of laying hens in converting rations into products in the form of eggs. Feed conversion ratio is closely related to feed consumption and egg production. Feed conversion ratio is influenced by several factors including chicken breed, growth speed, egg production, energy and protein content of feed, chicken health, environmental temperature, cage ventilation and ammonia content in the cage (Sjofjan, 2003). Feed efficiency can be used to see increased nutrient absorption and energy metabolism processes. Increasing feed efficiency has a positive impact because it can reduce production costs for farmers.

Income Over Feed Cost (IOFC)

Based on the results of the analysis of variance, it shows that the addition of RBPPP to the feed makes a very significant difference ($T < 0.01$) to the average IOFC. The research results showed that IOFC in treatment T3 produced the lowest value compared to other treatments, namely Rp(312.83 ± 13.47) per item, then sequentially the average feed conversion ratio from highest to lowest value for treatments T0, T1 and T2 was as follows Rp(683.83±13.20), (618.33±27.67) and (504.67±11.41) per item.

Based on DMRT IOFC T3, the level of RBPPP administration was 1.5 % lower than the control treatment or without the addition of RBPPP. This is because there are additional costs in adding RBPPP which are increasingly higher to the level of 1.5 % so that income decreases even though production performance is better than the control or T0 treatment which has lower feed costs. The best feed efficiency level at T2 has a low IOFC compared to T0 with the lowest efficiency level. This is suspected, even though the production performance of T2 is the best, on the other hand there are additional costs for RBPPP which are more expensive.

This IOFC value will be an indicator that can show whether a livestock business will make a profit or not with the addition of RBPPP. Ardiansyah, Tantalo and Nova (2013) stated that IOFC is greatly influenced by feed consumption, egg weight, feed prices and selling prices of livestock products. The higher the IOFC value, the better the chicken rearing is carried out, because the higher the IOFC means the income obtained from the sale of chicken eggs is also higher.

Effect of Treatment on Chicken Egg Yolk Cholesterol

Table 3 shows the effect of adding red beetroot pulp and peel powder to the feed on chicken egg yolk cholesterol. The results showed that the addition of RBPPP had no significant effect ($T > 0.05$) on egg yolk cholesterol, but numerically the addition of RBPPP could reduce egg yolk cholesterol at T3 by a level of 1.5 %, although not significantly.

Table 3. Effect of Adding RBPPP on Chicken Egg Yolk Cholesterol

Treatment	Egg Yolk Cholesterol (mg/g)
T0	232.22±28.84
T1	248.94 ± 31.22
T2	234.02 ± 39.93
T3	228.12 ± 16.85

Note: There are no significant differences between each treatment ($T > 0.05$).



The results showed that egg yolk cholesterol in treatment T3 produced the lowest value compared to other treatments, namely (228.12 ± 16.85) mg/g, then in sequence the average egg yolk cholesterol from highest to lowest value for treatments T1, T2 and T0 as follows (248.94 ± 31.22) , (234.02 ± 39.93) and (232.22 ± 28.84) mg/g. Egg yolk cholesterol levels in T1 and T2 with the addition of RBPPP were higher than in the control treatment or T0 without the addition of RBPPP. However, at T3 with a RBPPP level of 1.5 %, egg yolk cholesterol decreased to a lesser extent than at T0. This further indicates that the addition of RBPPP to a level of 1.5 % can reduce egg yolk cholesterol when compared to treatments with levels below and control treatments. The addition of RBPPP still plays a role in the process of digestion of food substances, although it is not optimal in reducing egg yolk cholesterol levels. The decrease in egg yolk cholesterol levels is thought to be due to the presence of several compounds such as betalains, flavonoids, saponins and tannins which can prevent LDL oxidation and work as inhibitors of the HMG-CoA reductase enzyme so that cholesterol synthesis decreases (Amila et al., 2021). This reduction in cholesterol levels is in accordance with research by Waiz (2023) that the addition of 25 ml of beet juice (*Beta vulgaris*) to drinking water can reduce total cholesterol levels, although relatively small from the control treatment of 131.59 mg/dL to 128.94 mg/dL in kadaknath chicken.

Effect of Treatment on the Blood Profile of Laying Hens

Table 4 shows the effect of adding red beetroot pulp and peel powder to the feed on the blood profile of laying hens. The results showed that the addition of RBPPP had a very significant effect ($T < 0.01$) on hemoglobin, erythrocytes and hematocrit. However, the addition of RBPPP had no significant effect ($T > 0.05$) on leukocytes.

Table 4. Effect of Adding RBPPP on the Blood Profile of Laying Hens

Variable	Treatment			
	T0	T1	T2	T3
Hemoglobin (g/dL)	10.39 ± 1.61^a	13.10 ± 1.33^{bc}	12.04 ± 1.22^{ab}	16.47 ± 2.17^d
Erythrocytes ($10^6/\text{mm}^3$)	2.26 ± 0.46^{ab}	3.11 ± 1.11^{abc}	2.25 ± 0.45^a	3.91 ± 0.61^c
Hematocrit (%)	26.84 ± 4.21^a	36.37 ± 1.27^{bc}	35.45 ± 1.31^b	55.95 ± 4.19^d
Leukocytes ($10^3/\text{mm}^3$)	1.76 ± 0.10	1.90 ± 0.11	1.75 ± 0.20	1.93 ± 0.48

Note: Different notations on the same line indicate very significant differences between treatments ($T < 0.01$).

Hemoglobin

Based on the results of analysis of variance, it shows that the addition of RBPPP in feed makes a very significant difference ($T < 0.01$) to the average hemoglobin of laying hens. The results of the study showed that the hemoglobin of laying hens in the control or T0 treatment (without the addition of RBPPP) produced the lowest hemoglobin value compared to other treatments, namely (10.39 ± 1.61) g/dL, then in sequence the average hemoglobin was from highest to lowest value for treatments P3, P1 and P2 as follows (16.47 ± 2.17) , (13.10 ± 1.33) and (12.04 ± 1.22) g/dL.

Based on DMRT, hemoglobin levels in treatments with the addition of RBPPP were at various levels higher than those in the control treatment or without the addition of RBPPP. This indicates that giving RBPPP can increase hemoglobin levels in the body of laying hens. This statement is strengthened by the content of vitamin B9 (folic acid) which can increase hemoglobin levels and prevent cases of anemia (Amila et al., 2021). The results of this study are higher than the research reported by Waiz (2023) which stated that the average hemoglobin level of kadaknath chickens with the addition of 0.02 % beetroot to drinking water was 11.05 g/dL. This is because there was an increase in the level of RBPPP provision during the research up to 1.5 %.

The average hemoglobin results in laying hens during the study ranged from 10.39-16.47 g/dL, which is slightly higher than normal hemoglobin levels in chickens which range between 10.2-15.1 g/dL (Samour, 2015). This normal hemoglobin condition indicates that RBPPP does not contain toxic substances that can cause lysis of red blood cells or interfere with the process of red blood cell formation (Teru, 2017). A normal hemoglobin count can be used as an indicator that adequate protein and amino acids are maintained. Apart from that, the flavonoid and antioxidant content in RBPPP can maintain the chicken's digestive tract well so that it can increase the feed absorption process, especially protein and minerals such as iron (Fe). The combination of protein and minerals in feed that is well absorbed can maintain the amount of hemoglobin in the blood, so that the amount of hemoglobin in the blood of laying hens is normal. The addition of RBPPP to a level of 1% in this study can maintain hemoglobin levels in normal conditions so that it does not have a negative impact on the physiology of laying hens.



Erythrocytes

Based on the results of analysis of variance, it shows that the addition of RBPPP in feed makes a very significant difference ($T < 0.01$) to the average erythrocytes of laying hens. The results showed that the erythrocytes of laying hens in treatment T2 with the addition of 1 % RBPPP produced the lowest erythrocyte values compared to other treatments, namely $(2.25 \pm 0.45) \times 10^6/\text{mm}^3$, then in sequence the hemoglobin average from the highest value to low for treatments T3, T1 and T0 as follows (3.91 ± 0.61) , (3.11 ± 1.11) and $(2.26 \pm 0.46) \times 10^6/\text{mm}^3$.

Based on DMRT, the erythrocyte content of the treatment with the addition of RBPPP was 1.5 % higher than the other treatments. However, at the level of 1 % RBPPP administration, the erythrocyte content value was lower than the control treatment or T0 and T1 with the addition of 0.5 % RBPPP. Numerically, there was an increase in the number of erythrocytes due to the administration of RBPPP, especially with the addition of RBPPP levels of 0.5 % and 1.5 %. The increase in the number of erythrocytes is thought to be due to the bioactive content and nutritional content in RBPPP which can stimulate the erythropoietin hormone to produce large numbers of erythrocyte cells. Beets work by stimulating blood circulation and helping build red blood cells because they contain folic acid and B12 which are important and necessary for the normal development of erythrocytes. Beets also cleanse and strengthen the blood so that the blood can carry nutrients throughout the body so that the number of red blood cells will not decrease (Amila *et al.*, 2021). The results of this study were higher than the research reported by Waiz (2023) which stated that the average erythrocyte content of kadaknath chickens with the addition of 0.02 % beetroot to drinking water was $3.72 \times 10^6/\text{mm}^3$. This is because there was an increase in the level of RBPPP provision during the research up to 1.5 %. The average erythrocyte results of laying hens during the study ranged from $2.25-3.91 \times 10^6/\text{mm}^3$ which is slightly lower than normal erythrocyte levels in chickens which range from $2.5-3.9 \times 10^6/\text{mm}^3$ (Samour, 2015). A normal number of erythrocytes can be used as an indicator that adequate protein and amino acids are maintained (Mozin, 2015). The addition of RBPPP at levels of 0.5 % and 1.5 % in this study can maintain erythrocyte levels in normal conditions so that it does not have a negative impact on the physiology of laying hens.

Hematocrit

Based on the results of the analysis of variance, it shows that the addition of RBPPP to the feed makes a very significant difference ($T < 0.01$) to the average hematocrit of laying hens. The results showed that the hematocrit of laying hens in the control or T0 treatment (without the addition of RBPPP) produced the lowest hematocrit value compared to other treatments, namely $(26.84 \pm 4.21) \%$, then in sequence the average hematocrit value was from highest to lowest for the treatment T3, T1 and T2 as follows (55.95 ± 4.19) , (36.37 ± 1.27) and $(35.45 \pm 1.31) \%$.

Based on DMRT, the hematocrit levels in the treatment with the addition of RBPPP were at various levels higher than those in the control treatment or without the addition of RBPPP. This indicates that giving RBPP can increase hematocrit levels in the body of laying hens. The results of this research are higher than the research reported by Waiz (2023) which stated that the average hematocrit level of kadaknath chickens with the addition of 0.02 % beetroot to drinking water was 41.33 %. This is because there was an increase in the level of RBPPP provision during the research up to 1.5 %. Hematocrit functions to measure the proportion of red blood cells (erythrocytes). An increase or decrease in hematocrit in the blood affects blood viscosity. The greater the percentage of hematocrit, the more friction that occurs in the blood circulation in the various blood layers and this friction determines viscosity, therefore blood viscosity increases as the hematocrit also increases (Ali, Ismoyowati and Indrasanti, 2013).

The average hematocrit results for laying hens during the study ranged from 26.84-55.95 %, which is higher than normal hematocrit levels in chickens which range between 24-43 % (Samour, 2015). This indicates that the addition of RBPPP up to a level of 1 % is able to maintain the hematocrit value in laying hens under normal conditions. Differences in hematocrit values in each study are possible due to differences in age, production level, rearing system and season (Ali *et al.*, 2013).

Leukocytes

Based on the results of analysis of variance, it shows that the addition of RBPPP in the feed has no significant effect ($T > 0.05$) on the leukocyte average of laying hens. The results showed that the leukocytes of laying hens in the T2 treatment with the addition of 1 % RBPPP produced the lowest leukocyte values compared to other treatments, namely $(1.75 \pm 0.20) \times 10^3/\text{mm}^3$, then in sequence the leukocyte averages from the highest to low for treatments T3, T1 and T0 as follows (1.93 ± 0.48) , (1.90 ± 0.11) and $(1.7 \pm 0.10) \times 10^3/\text{mm}^3$.



Based on DMRT, the leukocyte level of the treatment with the addition of RBPPP was 1.5 % higher than the other treatments. However, at the level of 1 % RBPPP administration, the leukocyte level was lower than the control treatment or T0 and T1 with the addition of 0.5 % RBPPP. Numerically, there was an increase in the number of leukocytes due to the administration of RBPPP, especially with the addition of RBPPP levels of 0.5 % and 1.5 %. The increase in the number of leukocytes is thought to be due to the role of active substances in the form of antioxidants and flavonoids contained in RBPPP which are able to suppress the development of pathogenic bacteria and protect the body against infectious agents against pathogenic microorganisms. Leukocytes are the active unit of the body's defense system by providing fast and strong defense against any infectious agent. This defense is carried out by destroying the attacking agent by the process of phagocytosis or by forming antibodies, so that metabolic processes in the chicken's body can be maintained properly. If the metabolic processes in the chicken's body are maintained in good condition, it will also affect the chicken's health condition.

The results of this study were lower than the research reported by Waiz (2023) which stated that the average leukocyte content of kadaknath chickens with the addition of 0.02 % beetroot to drinking water was $15.6 \times 10^3/\text{mm}^3$. The number of leukocytes that are different and have high fluctuations can occur due to stress conditions, high biological activity, nutrition and age. Other influencing factors are gender and environmental temperature (Arfah, 2015). The average erythrocyte results of laying hens during the study ranged from $1.75\text{-}1.93 \times 10^3/\text{mm}^3$ which is slightly lower than normal leukocyte levels in chickens which range between $1,900\text{-}9,500 \text{ cells}/\text{mm}^3$ (Weiss and Wardrop, 2010). The addition of RBPP at levels of 0.5 % and 1.5 % in this study can maintain leukocyte levels in normal conditions so that it does not have a negative impact on the physiology of laying hens.

CONCLUSIONS

Based on research regarding the addition of red beet powder (*Beta vulgaris L. Var. Rubra L*) in the feed of laying hens, it can be concluded that treatment with Red Beetroot Pulp Powder (RBPP), Red Beetroot Peel Powder (RBPP) and Red Beetroot Pulp and Peel Powder (RBPPP) had a very significant effect ($T < 0.01$) on the diameter of the inhibition zone for *Escherichia coli*, *Salmonella sp* and Lactic Acid Bacteria (LAB). The best part of the beetroot plant, namely RBPPP, has a medium category of inhibition of pathogenic bacteria on *Escherichia coli* and a strong category on *Salmonella sp*. even though it has a medium category of non-pathogenic bacterial inhibition in Lactic Acid Bacteria (LAB). The addition of RBPPP had a significant effect ($T < 0.05$) on feed consumption, had a very real effect ($T < 0.01$) on egg weight, HDP, egg mass, FCR, IOFC, hemoglobin, erythrocytes and hematocrit. However, there was no significant effect ($T > 0.05$) on egg yolk cholesterol and leukocytes. The addition of RBPP at the 1% level has the potential to reduce feed consumption and feed conversion ratio, increase egg weight, HDP and egg mass, even though the IOFC value is lower than the control treatment or without RBPPP. The addition of RBPPP at a level of 1.5 % has the potential to reduce egg yolk cholesterol levels compared to other treatments. The addition of RBPPP up to a level of 1 % is able to increase and maintain hemoglobin and hematocrit levels in normal conditions. Apart from that, the addition of RBPPP at levels of 0.5 % and 1.5 % is able to increase and maintain erythrocyte and leukocyte levels in normal conditions.

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