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EFFECT OF MICROCAPSULES OF NONI FRUIT EXTRACT ON ANTIOXIDANT LEVELS OF LAYER PHASE SENTUL CHICKEN

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Abstract

This study aims to determine the effect of the addition of microcapsules of noni fruit extract (Morinda citrifolia L.) on antioxidant levels in Sentul chickens in layer phase. A total of 40 female Sentul chickens aged 24 weeks were divided into five feed treatments with various levels of noni extract microcapsules: Control (P0), 50 mg/kg Zinc bacitracin (P1), 75 mg/kg (microcapsules of noni fruit extract) MEBM (P2), 150 mg/kg MEBM (P3), and 225 mg/kg MEBM (P4). The results showed that MEBM treatment significantly increased the levels of GR (Glutathione Reductase) and SD (Superoxide Dismutase). This study concluded that the use of microcapsules of noni fruit extract in Sentul chicken feed can increase antioxidant levels in the blood and improve livestock health. The obtained data indicate that the combined supplementation of MEBM and Zinc Bacitracin positively modulates blood cell indices, reflecting improved physiological and immune status in laying hens.

Key words: *blood biochemistry, microcapsules, noni fruit extract, Sentul chicken.*

INTRODUCTION

Local chickens cultivated in Indonesia include various species, one of which is Sentul Chicken from Ciamis Regency, West Java. Sentul chickens fall into the dual-purpose category as they have a faster growth rate than other local chickens, making them suitable as broilers. In addition, with a fairly high level of egg productivity, Sentul chickens also have the potential to be used as laying hens. As one of Indonesia's local poultry, Sentul Chicken production has great potential to support food security, especially as a source of animal protein. However, the metabolic efficiency of Sentul chickens in utilizing feed nutrients to support productivity, such as egg production, still needs to be optimized.

The genetic superiority of Sentul Chicken must be supported by good maintenance management in order to achieve maximum production performance. One important aspect in this case is feed management. The addition of certain compounds in the feed ration can be a solution to overcome nutritional and metabolic problems in poultry, so as to improve production performance. One potential alternative feed addition is the utilization of herbal plants that are generally safe, such as noni fruit (*Morinda citrifolia* L.) which contains various compounds such as alkaloids, flavonoids, and antioxidants. These compounds are useful as antimicrobials, rich in minerals and vitamins that can boost the immune system, improve appetite, and support nutrient absorption in the intestine, thus increasing ration efficiency.

Noni fruit extract containing active compounds such as flavonoids is expected to be an alternative to the use of antibiotics. Flavonoids act as antioxidants that are effective in destroying and neutralizing oxidants (Georgiev et al., 2014). Cell damage that occurs can be overcome by active substances from noni fruit, including helping to stabilize the growth of digestive tract organs (Mutia et al., 2017). This

study aims to determine the effect of the addition of noni fruit extract on antioxidant levels and its impact on health Sentul chicken.

MATERIALS AND METHODS

Animal, Housing and Location

A total of 40 female Sentul chickens used in this study were obtained from the Center for Poultry Development and Breeding (BPPTU) Jatiwangi. The chickens were intensively reared for 12 weeks, starting when they were 24 weeks old. They were divided into five feed treatment groups, with each treatment repeated four times, and randomly placed in 40 cage units. The average initial weight of Sentul chickens in the layer phase was 1341.1 grams, with a coefficient of variation of 14%.

The treatments included the level of microencapsules of noni fruit extract in the ration, as follows:

P0 = Basal Ration;
P1 = Basal Ration + 50 mg/kg Zinc bacitracin;
P2 = Basal Ration + 75 mg/kg MEBM;
P3 = Basal Ration + 150 mg/kg MEBM;
P4 = Basal Ration + 225 mg/kg MEBM.

Antioxidant Activity Test

The antioxidant activity of noni fruit extract was tested using the DPPH method with a UV-visible spectrophotometer. The DPPH method operates on the principle of observing the hydrogen atom capture reaction by DPPH from antioxidant compounds. The ability of noni fruit extract to donate hydrogen atoms or electrons is assessed based on the change in color of the DPPH solution in methanol from purple to clear. To prepare the DPPH solution, 6 mg of DPPH was dissolved in 200 mL of ethanol, resulting in a 30 ppm concentration. The percentage of free radical inhibition by DPPH (I %) was calculated using the formula:

$$I\ \% = [(\text{absorbance without sample} - \text{absorbance with sample})/\text{absorbance without sample}] \times 100\%.$$

The resulting inhibition percentages were plotted against the extract concentration to determine the IC50 value. The IC50 represents the concentration of the extract required to inhibit 50% of the DPPH free radicals, which was derived from the graph of extract concentration versus the percentage of DPPH inhibition (Kusmayadi et al., 2019).

Determination of Hematological Parameters in Sentul chickens

Blood samples were collected from the wing vein using sterile syringes and transferred into tubes containing anticoagulant (EDTA). The hematological parameters, respectively erythrocytes, leukocytes, and neutrophils, were measured using an automated veterinary hematology analyzer (Mindray BC-2800Vet) equipped with an avian mode. The analyzer determined cell counts by electrical impedance and optical scatter, with daily quality control and microscopic verification of flagged results. The erythrocyte and neutrophil count was expressed as $\times 10^6$ cells per microliter (µL) of blood. The result of total leukocytes was expressed as the number of leukocytes per microliter (cells/µL) of blood.

Data analysis

The data obtained were analyzed using analysis of variance, and differences between treatments were tested using Duncan's Multiple Range Test using SPSS IBM 25 software.

RESULTS AND DISCUSSIONS

Antioxidant Activity

The results of the antioxidant activity parameters SD, GR, and TAS in layer phase sentul chickens showed a significant increase (Table 1).

Table 1. Results of antioxidant activity testing in the layer phase of Sentul chicken

Parameters	Treatment				
	T0	T1	T2	T3	T4
SD, nmol.mg^{-1}	5.05^{a}	5.75^{b}	6.66^{c}	7.97^{d}	7.95^{d}
GR, nmol.mg^{-1}	5.66^{a}	7.69^{b}	7.77^{b}	10.57^{c}	10.57^{c}
TAS, nmol.mg^{-1}	13.21^{a}	15.45^{b}	15.97^{b}	17.47^{c}	17.48^{c}

$^{a, b, c}$Different notations on the same line indicate significant differences (P<0.05).
TAS: Total Antioxidant Status; GR: Glutathione Reductase; SD: Superoxide Dismutase.

Different notations indicate significant differences (P<0.05), the increase reached its peak at P3 and P4. SD (Superoxide Dismutase) increased from P0 (5.05) to P3 (7.97) and P4

(7.95) indicating optimal antioxidant enzyme activity in P3-P4 treatments. This indicates an improvement in free radical neutralization.

The GR (Glutathione Reductase) value also increased significantly between P0 (5.66) to P3-P4 (10.57), indicating an increase in glutathione cycle to support the antioxidant mechanism. The TAS (Total Antioxidant Status) parameter peaked at P3 (17.47) and P4 (17.48), reflecting the accumulation of total antioxidant capacity in these treatments.

Giving rations containing noni fruit extract (*Morinda citrifolia* L.) can increase the length of the digestive tract organs due to its antioxidant content. Noni fruit extract in the ration is able to prevent the growth of pathogenic microorganisms in the body of chickens, so that the function of digestive organs remains optimal and has increased. Flavonoids contained in noni fruit extract have various biological activities, such as antioxidant, antitumor, and immune system regulation effects (Vasil et al., 2014). In the context of animal production, flavonoids function to stimulate growth, improve reproduction, and strengthen the immune system (Surai, 2014). Flavonoids have anti-inflammatory properties through various mechanisms such as inhibition of regulatory enzymes and transcription factors that play an important role in the control of mediators involved in inflammation (Takeshi et al., 2015). Flavonoids are also powerful antioxidants capable of scavenging free radicals and reducing their formation (Chao et al., 2019; Syahrudin et al., 2012).

The presence of antioxidants plays a role in inhibiting oxidation processes that have the potential to damage oxidants and cause damage (Syahruddin et al., 2012). Antioxidants play a role in inhibiting the lipid peroxidation process and protecting cells from oxidative stress damage by preventing oxidative chain reactions (Gusti et al., 2024). In Yang et al. research (2023), showed that flavonoid administration tends to increase serum superoxide dismutase levels. Noni fruit extract is able to increase absorption in the intestine by expanding the field of nutrient absorption due to the length of the jejunum and villi organs so that the absorption surface area is wider (Purnata et al., 2018), so that the effectiveness of the absorption of food essence takes place so that it can improve the health and productivity of livestock (Putri et al., 2019).

Other research also states that flavonoids can reduce MDA and increase SD activity so as to improve the welfare of laying hens (Iskender et al., 2016). In the research of Ichsan et al. (2021), showed that the addition of noni fruit extract in the ration can increase hen day production and reduce the value of ration consumption and ration conversion even though it does not increase the average weight of eggs in Sentul chickens. Zn supplementation has antioxidant effects and is also able to reduce stress levels in chickens, which in turn improves the process of feed digestion. This is in line with the opinion of Gerzilov et al. (2015), which states that Zn supplementation can increase egg production and production performance, both of which are related to the anti-stress and antioxidant properties of Zn.

Hematology plays a crucial role in assessing the health status of poultry, particularly laying chickens. By analyzing various hematological parameters, veterinarians and poultry farmers can gain insights into the physiological condition of birds, which is essential for maintaining optimal health and productivity. Studies show temporary drops in erythrocytes, hemoglobin, and hematocrit during peak laying periods (e.g., week 6), reflecting metabolic stress from high egg production (Abdillah et al., 2021). Total leukocyte counts rise during infections or inflammation. For example, heat-stressed hens exhibit altered leukocyte profiles, indicating immunosuppression. Heterophils surge during bacterial infections, acting as primary defenders. Stressors like heat or poor housing amplify this response, compromising immunity (Hrabčáková et al., 2014; Mardhotillah et al., 2022; Kim et al., 2024).

Table 2 summarizes the hematological parameters of Sentul chickens during the laying phase. The evaluated parameters, erythrocyte, leukocyte, and neutrophil counts, serve as important indicators of the birds' hematopoietic activity, physiological adaptation, and immune status in response to the experimental treatments.

The erythrocyte count varied significantly across treatments ($P<0.05$), with the control group (P0) having the lowest mean value (2.70

± 0.096×10⁶/µL). The addition of Zinc Bacitracin in P1 resulted in a significant increase in erythrocyte count (3.84 ± 0.093×10⁶/µL), suggesting its role in improving blood health, possibly through enhanced gut health and nutrient absorption. In laying hens, higher erythrocyte levels indicate improved oxygen transport, which can enhance metabolic efficiency and overall production performance. This suggests that MEBM may support erythropoiesis up to a certain level, beyond which additional supplementation may not provide further benefits.

Table 2. Results of hematological parameters testing in the layer phase of Sentul chicken

Group	Erythrocytes (×10⁶/µL)	Leukocytes (/µL)	Neutrophils (×10⁶/µL)
P0	2.70 ±0.096[a]	174887.5 ±1557.9	3.125 ±0.25[a]
P1	3.84 ±0.093[d]	132310 ±682.4	2.2 ±0.24[c]
P2	3.11 ±0.069[b]	167950 ±2414.5	2.9 ±0.14[ab]
P3	3.33 ±0.113[c]	155300 ±2155.6	2.55 ±0.13[bc]
P4	3.71 ±0.140[d]	142850 ±2357.2	2.325 ±0.28[c]

[a, b, c, d]Different notations on the same line indicate significant differences (P<0.05).

The leukocyte count exhibited significant differences (P<0.05) among treatments. P0 had the highest leukocyte count (174887.5 ± 1557.9/µL), while P1 recorded a significant decrease (132310 ± 682.4/µL). This suggests that Zinc Bacitracin has a notable anti-inflammatory effect, possibly by reducing bacterial challenges in the gut, thereby lowering immune stress. The supplementation of MEBM resulted in a fluctuating trend, with P2 showing a significant increase (167950 ± 2414.5/µL) compared to P1 but not significantly different from P3 and P4 (P > 0.05). These results suggest that MEBM at moderate doses can enhance immune function in laying hens, but excessive supplementation may not provide additional immune benefits and could indicate a regulatory mechanism in immune response.

Neutrophil levels varied significantly across treatments (P < 0.05). P0 had a neutrophil level of 3.125 ± 0.25×10⁶/µL, while P1 showed a significant decrease (2.2 ± 0.24×10⁶/µL), suggesting that Zinc Bacitracin contributed to a reduced inflammatory response. In laying hens, maintaining optimal neutrophil levels is essential for immune defense, and these results suggest that MEBM at moderate levels can support immune function but does not provide additional benefits at higher concentrations.

The results indicate that dietary supplementation with microencapsulated noni fruit extract (MEBM) and Zinc Bacitracin significantly influences hematological parameters in laying hens. Zinc Bacitracin significantly increased erythrocyte count and reduced leukocyte and neutrophil levels, suggesting improved gut health and reduced immune stress (Kuźmicka et al., 2020; Skalny et al., 2021). The inclusion of MEBM exhibited an immunomodulatory role, with significant effects at moderate levels but diminishing returns at higher concentrations.

For laying hens, maintaining optimal hematological parameters is critical for productivity, as blood health directly affects oxygen transport, immune function, and overall physiological balance (Wlaźlak et al., 2023). The significant improvements in erythrocyte counts suggest that supplementation with MEBM could potentially enhance egg production by improving metabolic efficiency. However, the fluctuating leukocyte and neutrophil responses indicate that excessive supplementation may not yield additional benefits and could suggest a threshold effect in immune modulation.

Research shows that giving noni fruit flour can reduce cholesterol levels in eggs. This makes the eggs produced healthier and safer for human consumption (Santoso, 2016). These findings suggest that optimizing the dose of MEBM is crucial for achieving the desired immune and hematological benefits in laying hens. Further studies should analyze the statistical significance of these differences in greater detail, considering the interactions with other dietary components in commercial egg production systems.

CONCLUSIONS

MEBM treatment significantly increased the levels of GR (Glutathione Reductase) and SD (Superoxide Dismutase). Giving MEBM at

doses of 150 and 225 mg/kg showed the same results. This study concluded that the use of microcapsules of noni fruit extract in Sentul chicken feed can increase antioxidant levels in the body of livestock so as to improve livestock health.

The results demonstrate that dietary supplementation with microencapsulated noni fruit extract (MEBM) and Zinc Bacitracin exerts a significant effect on the hematological characteristics of laying hens.

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