



Effect of Combined Phytobiotic, Chromium and Betaine Supplementation on Performance, Egg Quality and Health Status in ISA Brown Laying Hens

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Abstract | This study aimed to evaluate the effects of a combined PCB (phytobiotic, chromium, and betaine) supplementation in the diet on the performance, egg quality, and health status of ISA Brown laying hens. A total of 2000 ISA Brown laying hens were used, divided into four treatment groups with five replicates each. The treatments were: T1 = control diet without supplementation, T2 = control diet supplemented with 0.05% PCB, T3 = 0.10% PCB, and T4 = 0.15% PCB. Data were analyzed using analysis of variance (ANOVA) in SPSS Statistics, and significant results were further examined with Duncan's multiple range test. The results showed that PCB supplementation at 0.05% and 0.10% significantly ($P < 0.05$) increased hen-day production, egg mass, eggshell color, and blood eosinophil levels, while also decreased ($P < 0.05$) daily feed intake, feed conversion ratio, and egg weight. In conclusion, 0.05% and 0.10% PCB increased egg production by 13% and improved feed efficiency by 9% in 82–87 week laying hens. Supplementation of PCB is an effective strategy for improving laying hen productivity and feed efficiency.

Keywords | Egg quality, Supplementation, Immunity, ISA Brown hens, Performance

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INTRODUCTION

The production period of ISA Brown strain laying hens is 80-90 weeks. Egg production typically increases around 22 weeks of age, peaks between 28 and 36 weeks, and gradually declines to about 55% by 82 weeks (ISA Brown Management Guide, 2019). This decrease in egg production is due to the feed consumed cannot be adequately digested. decreased production in laying hens can

be caused by heat stress, which negatively impacts physiological functions, reducing productivity, egg quality, and health status (Sugiharto, 2020). Heat stress can increase fat deposition in poultry (Emami *et al.*, 2021), can lead to oxidative stress and reduce egg production (Adib *et al.*, 2023). Besides that, the decrease in production is also caused by an age-related decline in organ function. One of the vital organ functions needed in metabolism in the body of live-stock is the liver (Nawab *et al.*, 2019).

The liver has a vital role in body activities such as glucose and lipid metabolism and detoxifying toxic substances (Nawab *et al.*, 2019). High-producing laying hens are susceptible to fatty liver hemorrhagic syndrome, a non-infectious metabolic disease influenced by nutrition, hormones, environment, and energy intake, especially in hens in the late production phase, leading to liver damage (Rozenboim *et al.*, 2016; Dey *et al.*, 2018). Fat accumulation in the liver will interfere with the performance process of the liver, the organ cannot work optimally. Some metabolic diseases in laying hens experience fatty liver syndrome, which can interfere with metabolic processes and egg production (Crespo and Senties-Cue, 2015; Buzafa *et al.*, 2015).

Feed additives in poultry consist of vitamins (Yusuf *et al.*, 2023), minerals, probiotics, and prebiotics. Farmers began to switch to using feed additives sourced from natural ingredients to replace antibiotics such as phytobiotics (Ogbuewu *et al.*, 2021). Phytobiotics can be a safe antibiotic replacement supplement and do not cause microbial resistance in humans; phytobiotics meet biological safety requirements for poultry and human health (Gilani *et al.*, 2021). Among the currently available non-antibiotic poultry feed additives and feeds, natural herbs and plants have been widely advocated due to their beneficial (Gautam, 2017). As a mineral Chromium can reduce the adverse effects of heat stress on performance and metabolic profile of laying hens. Chromium can be added to the diet to address heat stress-related depression in performance and metabolic profile (Sahin *et al.*, 2018).

Phytobiotics such as turmeric exhibit potent antibacterial activity and can optimize internal organ performance function in poultry (Kiczorowska *et al.*, 2017). Herbal ingredients such as turmeric could increase egg production in poultry because it can control lipids in the body so that it can increase poultry production, such as increased egg production and optimize the relative weight of digestive organs, among other things, improving the performance of digestive organs (Liu *et al.*, 2020). Betaine is the generic term for trimethylglycine, natural compound that can be found in plants and animals. due to its chemical structure, betaine exhibits the characteristics of a dipolar zwitterion with osmoprotective properties. Betaine provides its labile methyl groups for the methylation of homocysteine to methionine. The methyl groups are also required for the synthesis of several substances, such as creatine and carnitine, which play an important role in the oxidation of fatty acids (Eklund and Weiss, 2005). As laying hens age, their performance tends to decline due to reduced internal organ function and increased environmental stress. To address these challenges, the combination of phytobiotics, chromium, and betaine has been proposed as a potential solution to improve the performance of aging laying hens. Hence, we hypothesized of this study is that the combination of

phytobiotics, chromium, and betaine positively affects the performance of aging laying hens. This study aimed to evaluate the effects of combined PCB (phytobiotic, chromium, and betaine) supplementation on performance, egg quality, and health status in ISA Brown laying hens.

Table 1: Product specification of Citrus XL (in 1kg).

Material	Amount	Unit
Chromium Organic	100.00	mg
Phytobiotic	350.00	g
Betaine	200.00	g
Filler		qs

Note: ¹⁾ Nutricell Pacific. *Phytobiotic contains curcumin based on PT Nutricell Pacific.

MATERIALS AND METHODS

ANIMAL AND DIETS

The research was conducted at the Cisadane Pradana Farm, Semplak, Bogor Regency, West Java, Indonesia from December 2022 to February 2023. This research used 2000 ISA Brown laying hens aged 82-87 weeks, which were distributed according to a completely randomized design into four experimental treatments (T1, T2, T3, T4) of 100 birds each, then each treatment was randomly allocated to five subgroups of birds each as a replicate. Citrus XL from PT. Nutricell Pacific was used as feed additive in this research (Table 1). Dose selection refers in mechanical statistical calculations as well as in commerce. The treatments diets Phytobiotic (P), Chromium (C), Betaine (B) were: T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%. Birds were kept in the cages in opened house system that, one cage consisted of two birds. The cage was equipped with a feeding trough and a nipple drinker. The cage in this study is an open house, but the temperature and humidity in the cage are monitored continuously by using fans to help decrease the temperature if the temperature is too high, the fans are placed in the front, middle, and back areas of the cage. The heat stress index is calculated by adding temperature (F) with humidity. The average temperature produced during the research was $26.70 \pm 1.10^{\circ}\text{C}$ with a humidity of $83.30 \pm 6.79\%$ and heat stress index 163 ± 4.96 . In this study the birds were subjected to heat stress, where a heat stress index <150 (comfortable), 150-160 (decreased activity, increased water consumption, and decreased production), >160 (heat stress occurred). The mash diet was offered twice a day (07.00 and 13.00 h) with amount 116 g/bird/day. The nutrients content of the diets is presented in Table 2.

PERFORMANCE OF LAYING HENS

The performance of laying hens was measured from 82

- 87 weeks of age. Feed intake was calculated weekly by calculating the difference between the amount of feed given and the remaining feed. The feed conversion ratio was calculated from the total feed intake divided by the egg mass (Mousavi *et al.*, 2016). Hen day production (HDP) was calculated by summing the ratio of the number of eggs produced to the number of hens. Egg weight was calculated based on the results of weighing eggs every day during rearing, egg mass was calculated by multiplying egg production by egg weight, mortality obtained from the number of deaths during the study, and IOFC (Income Over Feed Cost) calculates the feed costs incurred and income from egg sales.

Table 2: Nutrients content of experimental diets.

Nutrient Content	Treatments			
	T1	T2	T3	T4
Water (maks%)	13.00	13.00	13.00	13.00
Ash (maks%)	14.00	14.00	14.00	14.00
Crude protein (min%)	17.00	17.00	17.00	17.00
Crude fat (min%)	3.00	3.00	3.00	3.00
Crude fiber (maks%)	7.00	7.00	7.00	7.00
Calcium (%)	3.25-4.25	3.25-4.25	3.25-4.25	3.25-4.25
Phosphorus (%)				
Phytase ≥ 400 FTU kg	0.45	0.45	0.45	0.45
Aflatoxin totally (maks μ kg ⁻¹)	50	50	50	50
Lysine (min%)	0.84	0.84	0.84	0.84
Methionine (min%)	0.42	0.42	0.42	0.42
Methionine+cystin (min%)	0.70	0.70	0.70	0.70
Threonine (min%)	0.58	0.58	0.58	0.58
Tryptophane (min%)	0.19	0.19	0.19	0.19

Note: ¹⁾ PT. Sreeya Sewu TBK with components Corn, Bran, Soybean Meal, Meat and Bone Meal, Olein, Palm Oil Meal, Wheat Bran, DDGS, Feather meal Feed Additives Used: Amino acids, minerals, vitamins, phytase enzyme, NSP enzyme, antifungal, antioxidant, organic acids. T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%.

EGG QUALITY

Egg quality measurements were conducted at the initial week, 2 weeks, and 6 weeks after treatment, the criteria for egg sampling was consistent across all hens. The percentage of egg component weight (%) was obtained by breaking the egg and separating the egg white and yolk; egg components, namely egg white, yolk, and shell, were weighed using a digital scale. The egg index was measured by measuring the length and width of the egg using a caliper before the egg was cracked. Haugh units were obtained by cracking the

egg on a glass table. The height of the egg white was measured with a caliper ± 1 cm from the yolk, and the Haugh unit was calculated using the Haugh formula (Haugh, 1937) $HU = 100 \log [H + 7.57 - 1.7W^{0.37}]$ where H was albumen thickness (mm), and W was the weight of the entire egg (g). yolk colour was compared using a yolk colour fan with 15 colour scores. Egg shell colour was measured using the Nutricell digital color meter. Eggshell thickness was measured on eggs cracked at the blunt end, sharp end, and center using a caliper. Eggshell strength was measured using the RH-DQ200 Egg Shell Strength Tester (Guangzhou RunHu Instruments Co., Ltd.). Eggshell porosity was measured using an eggshell scoring and grading system (Nutricell Eggs-Pert) to determine the % egg score indicating improved shell quality (Setyaningsih *et al.*, 2023).

IMMUNE ORGANS

Measurement of organ immunity was carried out at the end of the study. immune organs weighed individually. Immune organ measurements used 20 randomly selected chickens representing each treatment and replicate. This calculation by means of the percentage of the difference between the weight of the immune organs and the slaughter weight of the laying hens. Measurement of immunity organs (spleen and thymus) was computed considering the body weight at slaughter (Lan *et al.*, 2017).

BLOOD PROFILE ANALYSIS

The immune system in poultry can be identified from the hematological profile of the blood profile of the livestock. Hematology testing is not only used for diagnosis for treatment purposes, but also for health monitoring of treatment response. Blood haematology analysis consisted of red blood type count, white blood type count, haemoglobin, haematocrit, and white blood type differentiation. red blood type and white blood type calculating by the number of blood grains using a Neubauer haemocytometer (counting chamber) under a microscope. Haemoglobin levels were calculated using the Sahli method. The haematocrit value was determined by the microhematocrit method. The haematocrit value is determined by measuring the blood's % volume of leukocyte differentiation. Serial preparations were made, dried, then fixed with methanol for 5 minutes and stained with May Grunwald-Giemsa staining (Schalm, 1961) for 30 minutes. Counting was done on one hundred leukocyte cells using a microscope with 1000x magnification, including heterophils, basophils, eosinophils, lymphocytes, and monocytes. The results obtained are expressed as a percentage of each type of leukocyte. Lymphocyte ratio is obtained from the percentage of heterophils to lymphocytes.

STATISTICAL ANALYSIS

Data collected from this study were statistically analysed based on analysis of variance using the SPSS Statistics

software version 25 (2017). if significant differences were found ($p < 0.05$) between treatment groups, then further tested with Duncan's multiple range test. This further test was chosen because the study used laying hens of the same age, so that it could compare all treatments.

RESULTS AND DISCUSSION

PERFORMANCE OF LAYING HENS

Supplementation combination of PCB 0.05%, and 0.10% effect decreased ($P < 0.05$) feed intake, FCR (feed conversion ratio), egg weight, and egg mass, but did not show a significant effect ($P > 0.05$) on weight gain, and mortality (Table 3). However, the supplementation combination of PCB 0.05%, and 0.10% increased ($P < 0.05$) HDP (hen day production), egg mass, and IOFC. These results indicate that PCB feeding maximizes feed intake and is balanced by increased productivity, and improving income by the result of IOFC. The highest IOFC value was observed in hens receiving 0.10% PCB supplementation, yielding a profit of IDR 22,163.97 per bird.

Table 3: Performance of Isa Brown laying hens (aged 82-87 weeks).

Variables	Treatments			
	T1	T2	T3	T4
Feed Intake (g/bird)	115.09 ± 0.28 ^b	114.82 ± 0.41 ^{ab}	114.61 ± 0.20 ^a	115.15 ± 0.15 ^b
Feed conversion ratio	2.48 ± 5.63 ^b	2.29 ± 7.92 ^a	2.25 ± 6.76 ^a	2.45 ± 8.10 ^b
Hen day production (%)	71.09 ± 2.64 ^a	79.74 ± 2.23 ^b	80.19 ± 2.66 ^b	73.62 ± 1.87 ^a
Egg weight (g/egg)	63.60 ± 0.38 ^b	61.79 ± 0.54 ^a	62.11 ± 0.47 ^a	63.17 ± 0.30 ^b
Egg mass (g/bird ⁻¹)	46.11 ± 1.07 ^a	49.83 ± 1.56 ^b	50.62 ± 1.39 ^b	46.91 ± 1.40 ^a
Mortality (%)	0.20	0.60	0.80	0.40
IOFC (Rp/bird)	14606.14	21357.86	22163.97	17762.86

Note: T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%. ^{a-b}Means in the same row with different superscripts differ significantly ($p < 0.05$). *Egg price on Farm IDR 26,000.00 per kg and feed price IDR 6,900.00 per kg.

EGG QUALITY

Supplementation combination of PCB 0.05%, 0.10%, and 0.15% increased effect eggshell colour ($P < 0.05$), but did not show a significant effect ($P > 0.05$) on shape index, albumen index, yolk index, percentage of albumen and egg yolk, haugh unit, yolk colour, eggshell thickness, eggshell strength, and eggshell porosity (Table 4).

Table 4: Egg Quality of Isa Brown laying hens (aged 82-87 weeks).

Variables	Treatments			
	T1	T2	T3	T4
Shape index	0.76±0.00	0.76 ± 0.00	0.76 ± 0.00	0.75±0.01
Albumen index	0.90±0.03	0.88 ± 0.06	0.91 ± 0.03	0.87±0.05
Yolk index	1.00±0.04	1.02 ± 0.02	1.00 ± 0.01	0.99±0.22
Albumen (%)	55.83±3.54	58.06±1.85	57.06±2.48	57.69±2.06
Yolk (%)	32.92±3.49	30.96±1.78	31.86±2.37	31.62±1.99
Shell (%)	11.34±0.55	10.98±0.23	11.08±0.36	10.68±0.50
Haugh unit	89.67±4.54	87.75±3.51	90.37±3.89	90.06±3.07
Yolk color	8.40±0.54	8.80±0.44	8.80±0.44	8.60±0.89
Eggshell color	10.80 ± 0.44 ^a	14.60 ± 0.54 ^c	14.20 ± 0.83 ^c	12.40 ± 0.54 ^b
Eggshell thick-ness (mm)	0.37±0.01	0.36 ± 0.02	0.37 ± 0.00	0.36 ± 0.01
Eggshell strength (kgf)	1.66±0.31	1.58 ± 0.28	1.47 ± 0.29	1.10 ± 0.35
Eggshell porosity	2.30 ± 0.32	2.40 ± 0.51	2.35 ± 41.83	2.60 ± 0.37

Note: T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%. ^{a-b}Means in the same row with different superscripts differ significantly ($p < 0.05$).

IMMUNE ORGANS

Supplementation combination of PCB in the diet of ISA Brown laying hens at the age of 82 – 87 weeks did not show a significant effect ($P > 0.05$) on the percentage of immune organs (Table 5).

Table 5: Immune organs of Isa Brown laying hens (aged 82-87 weeks).

Variables	Treatments			
	T1	T2	T3	T4
Thymus (%)	0.22±0.03	0.18±0.03	0.18±0.03	0.19±0.06
Spleen (%)	0.15±0.08	0.14±0.05	0.11±0.01	0.11±0.02

Note: T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%.

BLOOD PROFILE ANALYSIS

Supplementation combination of PCB 0.10% effect decreased ($P < 0.05$) on the percentage of eosinophils and ratio H/L (heterophiles/lymphocytes) and did not show a significant effect ($P > 0.05$) on percentage of Hemoglobin, hematocrit, erythrocytes, leukocytes, lymphocytes, monocytes, heterophils, and basophils (Table 6).

Table 6: Blood profile of Isa Brown laying hens (aged 82-87 weeks).

Variables	Treatments			
	T1	T2	T3	T4
Hemoglobin (g%)	9.56 ± 0.93	9.48 ± 0.52	9.12 ± 0.88	9.56 ± 0.93
Hematocrit (%)	23.60 ± 3.04	24.40 ± 1.51	25.00 ± 2.82	23.60 ± 3.04
Erythrocytes (10 ⁶ /mm ³)	2.60 ± 0.64	2.14 ± 0.32	2.32 ± 0.39	2.60 ± 0.64
Leukocytes (10 ³ /mm ³)	49.76 ± 13.69	46.12 ± 3.00	29.54 ± 13.13	49.76 ± 13.69
Lymphocytes (%)	50.21 ± 0.88	49.73 ± 1.34	56.85 ± 1.46	50.21 ± 0.88
Monocytes (%)	4.49 ± 1.77	3.42 ± 1.58	2.96 ± 1.00	4.49 ± 1.77
Heterophils (%)	32.78 ± 2.50	31.89 ± 2.46	29.35 ± 2.04	32.78 ± 2.50
Basophils (%)	2.18 ± 0.46	1.86 ± 0.65	1.57 ± 0.73	2.18 ± 0.46
Eosinophils (%)	10.33 ± 1.27 ^a	13.08 ± 1.34 ^b	9.24 ± 0.88 ^a	10.33 ± 1.27 ^a
Ratio H/L	0.65 ± 0.06 ^b	0.64 ± 0.05 ^b	0.52 ± 0.04 ^a	0.47 ± 0.15 ^a

Note: T1 = diet without supplementation (Control), T2 = Control diet supplemented with PCB 0.05%, T3 = Control diet supplemented with PCB 0.10%, T4 = Control diet supplemented with PCB 0.15%. ^{a-b}Means in the same row with different superscripts differ significantly ($p < 0.05$).

PERFORMANCE OF LAYING HENS

The results of this study show that supplementation combination of PCB has a significant impact on the performance of laying hens (Table 3). Curcumin can increase egg production in poultry because it can control lipids in the body so as to increase poultry production, such as increased egg production, so the addition of PCB can optimize feed intake with an increase in the amount of production. Supplementation combination of PCB effect decreased feed intake of 114.61 g this result is similar to Cui *et al.* (2022) where curcumin supplementation of 50 mg/kg - 150 mg/kg resulted in feed intake of 114.76 - 114.99 g. However, this result is lower than the standard feed intake of 82-87 weeks of Isa brown laying hens, which is 115 g (ISA Brown Management Guide, 2019).

This condition was also supported by the FCR. Less feed intake and more egg production can decrease FCR value. Phytobiotic such curcumin and betaine, which curcumin can help livestock and poultry improve their physiological production status by regulating feed intake and appetite, promoting the browning of white adipose tissue, inhibiting fat synthesis, and maintaining livestock health in condi-

tions of a high heat stress index (Ejaz *et al.*, 2009; Kang *et al.*, 2011; Xie *et al.*, 2019; Br Pakpahan *et al.*, 2023). The addition of turmeric or cinnamon or their mixture to diets was actually hoped to be able to enhance the metabolism that later on could improve the feed efficiency (Al-Sultan and Gameel, 2004). In which the osmoregulatory function of betaine supports intestinal health and enhances cell activity, thereby improving nutrient digestibility (Won *et al.*, 2023). Some studies have also shown the positive effects of betaine on the growth performance of broilers under stress conditions, such as coccidiosis, heat exposure, and transportation (Chen *et al.*, 2020). Supplementation combination of PCB 0.10% effect increased hen day production of 80.19% higher than the standard HDP of 82-87 weeks of Isa brown laying hens, which is 76.6% - 79.7% (ISA Brown Management Guide, 2019). Chromium increases circulating insulin concentrations, regulating glucose levels by acting as an insulin cofactor and enabling proper metabolic transformations of carbohydrates, proteins, and lipids towards the anabolic side (Feng *et al.*, 2021).

Supplementation combination of PCB in the diet had a lower egg weight but a higher egg mass than the control diet. The results positively correlate with HDP because egg mass production will be affected by egg production, weight, and heat stress index (Vercese *et al.*, 2012). Supplementation of combination of PCB 0.10% effect decreased an egg weight 62.11 g, however increased egg mass 50.62 g, lower than the standard egg weight of 82 - 87 weeks of isa brown laying hens, which is 64.5 - 64.6 g and egg mass 49.5 - 51.4 g (ISA Brown management guide, 2019). The weight of the albumen in the egg influences egg weight and egg mass. The albumen consists of protein contained in the diet. This study used a diet with the same protein content. Other factors affecting egg weight include genetics, egg production, protein, and amino acids (especially methionine), which play an essential role in controlling egg size (Leeson and Summers, 2005). The results of this study are in line with Ayeni *et al.* (2020), where the addition of 3% curcumin to 64 weeks ISA Brown laying hens produced an egg weight of 62.92 g lower than the control feed of 64.99 g. Combination of PCB contains phytoestrogens such a curcumin, phytoestrogens are herbal compounds that mimic estrogen and induce estrogenic effects that can affect oviduct development (Saleh *et al.*, 2021).

The most mortality in this study was obtained when using a dose of as 0.10% diet (Table 3). Cannibalism occurs because there are two hens in one cage, which hens have a natural nature to peck, this happens because of the unequal weight factor in one cage, even though this has been avoided by equalizing the weight of hens in each cage, but over time, this factor reappears. Mortality in this study was attributed to cannibalism and aging (Weeks *et al.*, 2016;

Son *et al.*, 2022). Income over feed cost (IOFC) is a concept to determine profit as an early indicator of a livestock business analysis. The calculation of income over feed costs can be seen from the difference between income and feed costs during the livestock rearing period (Bach, 2023). The cost of feed intake in each treatment is relatively the same, but the best IOFC value was achieved in laying hens with supplementation combination of PCB as much as 0.10% diet (T3) with a profit of Rp 22,163.97/bird, while the control diet was Rp 14,606.14/bird. Which Egg price on Farm IDR 26,000.00/kg and feed price IDR 6,900.00/kg This shows that supplementation combination of PCB increases production and gets more profit for farmers.

EGG QUALITY

This study's results show that supplementation combination of PCB does not significantly affect the egg quality of laying hens (Table 4). egg index in the treatment diet gives almost the same results as the control diet, which is in line with the control diet. The addition of PCB supplementation does not have the content to significantly improve egg quality, this can be seen from the absence of significant changes in treatment and control eggs. This is in line with Dalal *et al.* (2018), who reported that turmeric powder supplementation had no significant effect on external and internal egg quality in laying hens compared to the control group. The percentage of egg weight with supplementation combination of PCB does not give significant results. This is because the study used the type of diet with the same protein content. Ratriyanto *et al.* (2018) explain that egg weight is influenced by the weight of the albumen and yolk, which consists mainly of protein. Reduced protein and amino acids in feed during the growth phase can inhibit sex maturity and reduce egg size (Siahaan *et al.*, 2013).

The haugh unit values were not significantly different due to the age of the hens, and the same protein and energy content in the feed in each treatment resulted in the haugh unit in this study. Haugh unit is a conventional unit of internal egg quality predictor, as egg quality is determined by albumen height. As laying hens age, Haugh units decrease due to moisture loss and protein breakdown within the albumen (Pires *et al.*, 2021). Egg yolk color is determined by the content and pigmentation profile of carotenoids contained in the feed (Bovšková *et al.*, 2014). In this study, the yolk color gave insignificant results. This study is in line with the results of Ismoyowati *et al.* (2018).

The external and internal characteristics of an egg, such as shell strength, shell color, shell thickness, etc. are crucial as they influence the consumer's acceptability and price of the egg (Tabeeekh, 2011). Shell color intensity is an important marketing measure in brown eggs, and preference is determined by region, with some markets preferring dark

eggs and others preferring light brown eggs (Odabasi *et al.*, 2007). The results of this study showed that supplementation combination of PCB had a significant impact on egg-shell color (Table 4). The main factors that can affect egg-shell color are biliverdin and porphyrin pigments (Wang *et al.*, 2009), genetics, stress, age, and disease (infectious bronchitis) (Aygün, 2014). Hens that produce eggs with brown-colored shells produce only protoporphyrin compounds (Clunies *et al.*, 1992). In addition to being affected by the type of pigment, eggshell color can also be affected by the concentration of egg color pigments and eggshell structure (Hargitai *et al.*, 2011). However, the variables of shell thickness, shell strength, and porosity had no significant effect. This is due to the absence of differences in calcium content in the treatment and control diets. Eggshell quality is associated with increased calcium availability and digestibility (Mikulski *et al.*, 2012). The improvement in eggshell quality is mainly due to the increased feed intake of laying hens, which liberates calcium in the serum combined with plasma protein or other components so that there is enough Ca^{2+} in the blood to participate in eggshell formation (Kosti *et al.*, 2020).

IMMUNE ORGANS

The immune organs of birds can be specialized into peripheral immune organs, e.g., the Spleen and cecum tonsil, and central immune organs, e.g., Thymus, bursa, and bone marrow, based on their structure and function and their function (Song *et al.*, 2021). This study had no significant effect on the percentage of spleen and thymus (Table 5). The spleen and thymus are important lymphoid organs that play an important role in the immune response to pathogenic infections. B-cell-mediated humoral immunity, T-cell-mediated immunity, and phagocytosis are the main functions of the spleen (Hu *et al.*, 2021). Biochemically, the immunostimulant activity is caused by the antioxidant content contained in curcumin, which acts as a trigger for the immune system. Antioxidants can prevent cell damage, stabilize cell wall structure, and increase endurance, and can also be influenced by the age of the laying hens (Varalakshmi *et al.*, 2008). In addition, the spleen is a storage and maturation site for monocytes. In the event of infection, these monocytes leave the spleen and begin to transfer to the area of inflammation (Filip *et al.*, 2009).

BLOOD PROFILE ANALYSIS

The results of this study show that supplementation combination of PCB does not significantly affect the blood profile analysis of laying hens (Table 6). Haemoglobin is an important component of erythrocytes, the acid buffer of blood, and an intermediary for oxygen transport throughout the body (Comito *et al.*, 2007). Various factors, including oxygen availability in the blood, can influence haemoglobin values. The average haemoglobin levels in this study ranged from 8.76% - 9.56 g%, which is within normal

limits. Measuring haematocrit value determines the body's health status (Virden *et al.*, 2007). The average haematocrit levels in this study ranged from 23.60% - 26.40 g%, which is within normal limits. The haematocrit value indicates the sustainability of transporting oxygen and nutrients in the blood (Etim *et al.*, 2013). This suggests that bioactive compounds from *Curcuma longa* are beneficial in regulating poultry thermotolerance. Erythrocytes are the most significant component of blood and play an important role in the transport of oxygen and nutrients needed by the body (Virden *et al.*, 2007).

Leukocytes are one of the body's defence mechanisms that function to produce antibodies that can resist viruses, fungi, bacteria, and parasites that cause diseases that enter the body (Davis *et al.*, 2008). According to Nawab *et al.* (2020), the administration of curcumin has no significant effect on the number of leukocytes due to the formation process of pluripotent hematopoietic stem cells requires complex factors in addition to nutritional adequacy. Lymphocytes play a role in humoral and cellular immunity, generally located in tissues or the spleen. The average lymphocyte levels in this study ranged from 49.73% - 56.85%, and normal lymphocytes in chickens are 20% - 65% (Gross and Siegel, 1983). In addition, the factor that affects blood profile is water consumption, in this study it was confirmed that water consumption was adjusted and ad libitum, so that chickens did not lack water consumption.

In this study, the number of eosinophils in the blood increased in treatment (T2) and then decreased in (T3) and (T4). The increase in the number of eosinophils was due to the presence of curcumin compounds, which are immunostimulants by increasing phagocytic activity where, in this case, the function of eosinophils is detoxification (Yhoan *et al.*, 2020). Some of the biological activities of turmeric include anti-inflammatory, antioxidant, anticoagulant, antidiabetic, antibacterial, antifungal, antiviral, and antiprotozoa (Liu *et al.*, 2020). This decrease is due to the anti-inflammatory substances contained in turmeric, causing eosinophil production to be reduced. According to Gross and Siegel (1983), stress levels in poultry can be determined by the H/L ratio, birds with H/L ratios of 0.20 (low stress), 0.50 (moderate stress) and 0.80 (high stress). In this study, the average H/L ratio was 0.47 - 0.65. This shows that laying hens suffer moderate stress during the study. Stress in laying hens due to being locked in cages for long periods (Shini *et al.*, 2019).

CONCLUSIONS AND RECOMMENDATIONS

PCB supplementation at 0.05% and 0.10% in diet increased egg production by 13% and feed efficiency by 9% in 82-87 week old laying hens, thereby benefiting farmers

in increasing egg sales. The optimal PCB supplementation level to achieve the best production performance was at a dose of 0.10% diet. This result was obtained in ISA Brown laying hens that experienced moderate stress due to the open cage system. Further research is needed to determine the combination of PCB supplementation in other strains with a closed cage system, in order to find out whether different cage conditions can increase egg production more until the laying hens are 100 weeks old.

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AUTHOR'S CONTRIBUTIONS

Renaldy Wira Kusuma: Conducted experiments, curated research data, data analysis, wrote the original script, drafted, edited the script and prepared research materials.

Widya Hermana: Designed the experimental design, drafted, reviewed, formal analysis and edited the script.

Sumiati: Designed the experimental design, drafted, reviewed, formal analysis and edited the script.

Wira Wisnu Wardani: Drafted, reviewed, formal analysis and edited the script.

Nofitra Dewi Suparno Putri: Drafted, edited the script, prepared research materials and curated research data.

Ilham Akbar: Drafted, edited the script, prepared research materials and curated research data.

ETHICAL APPROVAL

All experimental procedures were approved by the Animal Ethics Committee of IPB University by guidelines for the care and use of animals in the study (Number: 058-2023 IPB).

CONFLICT OF INTEREST

The authors report no conflict of interest.

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