



Effect of Novel Feed Additive on Egg Production and Blood Parameters of Hens during Post-Peak Production

(Pengaruh *novel feed additive* terhadap produksi telur dan parameter darah ayam petelur pasca puncak produksi)

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ABSTRACT. The present study evaluated the effect of feed additives containing *Averrhoa bilimbi* L. fruit filtrate, wheat bran, and *Saccharomyces cerevisiae* on egg production and blood parameters of laying hens during post-peak production. The experiment was carried out according to a completely randomized design with 224 hens (80 weeks old). The hens were randomly distributed to 4 treatments and 7 replications. The treatments included T0 (basal feed), T1 (basal feed + 0.25% additive), T2 (basal feed + 0.5% additive), T3 (basal feed + 1% additive). Feed intake was recorded daily, body weight was determined weekly, and blood was collected on day 35 of the experiment. The variables measured included feed intake, feed conversion ratio, hen day production, egg weight, yolk index, albumin index, eggshell thickness, yolk color, and complete blood counts. Results showed that hen-day production decreased ($P<0.05$) as the level of additives increased in the feed. Dietary inclusion of additive at 0.25% increased ($P<0.05$) eggshell thickness. The feed conversion ratio tended ($P=0.08$) to be better in hens receiving additives at 0.25%. Compared to control, additive at 0.5% decreased ($P<0.05$) red blood cell distribution width-standard deviation (RDW-SD) and red blood cell distribution width-coefficient variation (RDW-CV) of laying hens. In conclusion, novel feed additives containing *A. bilimbi* L. fruit filtrate, wheat bran, and *S. cerevisiae*, particularly at 0.25% of the diet improved hen day egg production, eggshell thickness, and feed conversion ratio.

Keywords: laying hens, organic acids, prebiotics, probiotics

ABSTRAK. Penelitian ini mengevaluasi pengaruh imbuhan pakan yang mengandung filtrat buah belimbing wuluh, dedak gandum dan *S. cerevisiae* terhadap produksi telur dan parameter darah ayam petelur selama pasca produksi puncak. Penelitian dilaksanakan berdasarkan rancangan acak lengkap (RAL) menggunakan 224 ekor ayam petelur (umur 80 minggu). Ayam-ayam tersebut didistribusikan ke 4 kelompok perlakuan dan 7 ulangan. Perlakuan tersebut meliputi T0 (pakan basal), T1 (pakan basal + aditif 0,25%), T2 (pakan basal + aditif 0,5%), T3 (pakan basal + aditif 1%). Konsumsi pakan dicatat setiap hari, bobot badan ditimbang setiap minggu dan darah diambil pada hari ke-35 penelitian. Parameter yang diamati dalam penelitian meliputi konsumsi pakan, rasio konversi pakan, produksi telur harian, berat telur, indeks kuning telur, indeks albumin, ketebalan cangkang telur, warna kuning telur dan profil darah lengkap. Hasil penelitian menunjukkan bahwa *hen day production* menurun ($P<0,05$) seiring dengan peningkatan level aditif dalam pakan. Aditif pakan pada level 0,25% meningkatkan ketebalan cangkang telur ($P<0,05$). Rasio konversi pakan cenderung lebih baik ($P=0,08$) pada ayam petelur yang menerima aditif sebesar 0,25%. Dibandingkan dengan kontrol, aditif 0,5% menurunkan RDW-SD dan RDW-CV ($P<0,05$) ayam petelur. Sebagai kesimpulan, imbuhan pakan yang mengandung filtrat buah belimbing wuluh, dedak gandum dan *S. cerevisiae* khususnya pada level 0,25% meningkatkan produksi telur ayam, ketebalan cangkang telur dan rasio konversi pakan.

Kata kunci: ayam petelur, asam organik, prebiotik, probiotik

INTRODUCTION

In general, egg production falls after laying hens reach the peak of production. Prophylactic antibiotics are frequently used in feed to delay the rate of egg production fall following the peak of production (Salim *et al.*, 2018). If these antibiotics are not absorbed by the hens, the use of antibiotics is not harmful to the product. Unfortunately, this is not always the case. As a result, antibiotic residues may accumulate in eggs, which can be harmful to consumers (Mund *et al.*, 2017). For this

reason, the use of prophylactic antibiotics in laying hens has been banned in most countries, including Indonesia. This prohibition may, however, have an impact on decreasing egg production and increasing morbidity and mortality in laying hens (Mund *et al.*, 2017). For the food safety reason and sustainable egg production, the attempt to find an alternative to prophylactic antibiotics is therefore crucial.

The use of organic acids, probiotics, and prebiotics has recently been implemented in the poultry industry as an alternative to prophylactic antibiotics (Sugiharto, 2016). With regard particularly to organic acids and probiotics, Pratama *et al.* (2021) used fermented *Averrhoa bilimbi* L. fruit filtrate rich in citric acid and lactic acid bacteria through drinking water. The

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treatment improved feed efficiency, intestinal ecology, and physiological condition of broiler chickens. In terms of prebiotics, there are many sources of prebiotics that can be used to improve the performance and health of poultry, one of which is wheat bran. A study in laying hens showed that the use of wheat bran in feeds improved egg production, egg weight, egg mass, feed conversion ratio, and egg yolk color index (Saleh *et al.*, 2020). *Saccharomyces cerevisiae* has long been used as a probiotic in poultry. In laying hens, *S. cerevisiae* has been reported to improve the laying performance of aged hens as well as increase activities of intestinal digestive enzymes and health (Zhang *et al.*, 2020).

With the aim to gain the synergistic and complementary effects among organic acid, probiotic, and prebiotic, *A. bilimbi* L. fruit filtrate, wheat bran, and *S. cerevisiae* were combined and used as feed additives for laying hens in this current study. This study was actually expected to produce feed additives that can be employed to maintain egg production and physiological conditions of laying hens during post-peak production. This is essential for the farmers as they can increase egg production per hen over their lifetime. Overall, the present investigation aimed to evaluate the effect of feed additives containing *A. bilimbi* L. fruit filtrate, wheat bran, and *S. cerevisiae* on egg production and blood parameters of laying hens during post-peak production.

MATERIALS AND METHODS

Preparation of Feed Additive

The production of feed additives was carried out according to the methods outlined by Sugiharto *et al.* (2021). Ripe *A. bilimbi* L. fruit was collected from the garden around the campus of Universitas Diponegoro, Semarang. The fruit was washed thoroughly with running water and then blended using an electrical blender. The juice was shifted using a cheesecloth to obtain the filtrate of *A. bilimbi* fruit. Wheat bran was purchased from a local supplier in Semarang. The wheat bran was added with distilled water (3:1, g:mL), stirred until homogeneous, autoclaved at 121°C for 15 minutes, and then allowed to cool at room temperature. *A. bilimbi* L. fruit filtrate was added to the autoclaved wheat bran (4:1, mL:g) and fermented anaerobically using an anaerobic jar at 38°C for 2 days. The fermented wheat bran was sun-dried, ground into meal, and subsequently added (2:1, g:g) with *S. cerevisiae* (Angle Yeast

Co. Ltd- Hubei, China) before being administrated to laying hens. The characteristics of the additive are described in Sugiharto *et al.* (2021).

In Vivo Study

The study employed a number of 224 laying hens (strain Isa Brown, average body weight of 1938 ± 92 g) 80 weeks old. The study was designed based on a completely randomized design with 4 treatments and 7 replications. Each experimental unit contained 8 laying hens. The treatments applied included T0 (basal feed), T1 (basal feed + 0.25% additive), T2 (basal feed + 0.5% additive), T3 (basal feed + 1% additive). The feed used during the study was a commercial feed (Starfeed L25MS) consisting of corn, soybean meal, meat, and bone meal. The feed contained 10.17% moisture, 17.96% dry matter crude protein, 2.99% dry matter crude fat, 8.79% dry matter crude fiber and 11.53% dry matter ash. The additives were given to laying hens according to their respective treatment groups through the feed. The additives were added 'on top' to the feeds and then thoroughly mixed before being given to the hens. Laying hens were reared in an open-sided layer house with a battery cage system, each containing 2 hens for 35 days. Feed consumption was recorded every day, while body weight was determined every week during the study. Egg production was recorded daily from the morning and evening egg collection.

Blood was taken from the wing veins of laying hens from each replication (seven samples from each treatment). A 3 mL of blood was then put into a tube containing ethylene diamine tetra acetic acid (EDTA) to prevent blood clots. The complete blood profile of laying hens was determined with a hematological analyzer based on the manufacturer's protocol (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). Data on egg quality were obtained from egg collections carried out in the last three days of the study. One egg was taken at random from each cage. After being weighed, the eggs were used to measure egg quality parameters. The final data obtained was the average result of three eggs for three days of collection.

The albumen index was obtained by measuring the length, width, and height of the albumen using a micrometer with an accuracy of 0.1 mm. The albumen index was calculated by the formula according to Sözcü *et al.* (2021) as follows: $a/\{0.5 (b_1+b_2)\}$, where a: height of albumen (mm), b_1 : diameter length of albumen 1

(mm) and b_2 : short diameter of albumin 2 (mm). The yolk index was obtained by measuring the diameter and also the height of the yolk using a micrometer with an accuracy of 0.1 mm. The yolk index was calculated by the formula according to Ozenturk and Yildiz (2020) as follows: a/b , where a : height of egg yolk (mm) and b : diameter of egg yolk (mm). The Haugh unit was obtained by weighing the egg weight and then measuring the albumen height using a micrometer. Haugh units were calculated using the formula according to Ozenturk and Yildiz (2020) as follows: $100 \log (H + 7.57 - 1.7 W^{0.37})$, where H : egg albumen height (mm) and W : egg weight (g). The yolk color was measured by assessing the color of the yolk using a color hue with a color scale of 1–15. The thickness of the eggshell was measured by separating the eggshell from the shell membrane, then cleaning and measuring the thickness of the shell at the pointed end, middle, and blunt end using a micrometer with an accuracy of 0.1 mm, and then calculating the average. The data collected during the study were analyzed based on analysis of variance (ANOVA) at the significant level of 5%.

RESULTS AND DISCUSSION

Data in our present study showed no substantial effect ($P>0.05$) of the treatments on feed intake, egg weight, yolk index, albumin index, and yolk color (Table 1). Although did not differ compared to the control, it was apparent that the hen day production decreased ($P<0.05$) as the level of additive increased in the feed. The reasoning for this condition was not known, but the conditions that are too acidic in the digestive tract due to the additive inclusion in diet may lead to acidic stress that can impair egg production. Although this phenomenon has not been observed in laying hens, a study in broiler chickens by Melaku *et al.* (2021) confirmed that dietary organic acid resulted in a substantial decrease in feed palatability, performance, and feed digestibility, as well as liver dysfunction. Research with different results has shown that administering citric acid at a rate of 30 g/kg in broiler diets can have positive effects. At this level, citric acid has been found to improve the growth, nutrient digestibility, and physiological conditions of broiler chickens.

Table 1. Laying performance and egg production of hens

Parameters	T0	T1	T2	T3	SEM	P value
Feed intake (g/day)	107	106	109	105	1.08	0.58
Feed conversion ratio	2.01	1.88	2.07	2.20	0.04	0.08
Hen day production (%)	83.3 ^{ab}	92.4 ^a	81.7 ^b	77.7 ^b	1.89	0.03
Egg weight (g)	63.4	64.4	64.9	63.5	0.48	0.71
Yolk index	0.40	0.40	0.43	0.44	<0.01	0.26
Albumin index	0.29	0.11	0.12	0.13	0.04	0.44
Eggshell thickness (mm)	0.36 ^b	0.52 ^a	0.42 ^{ab}	0.42 ^{ab}	0.02	0.03
Colour of yolk	9.20	9.20	9.60	10.0	0.13	0.10

^{a,b}In the same row, the means marked by superscript letters differ significantly ($P<0.05$)

T0: basal feed, T1: basal feed + 0.25% additive, T2: basal feed + 0.5% additive, T3: basal feed + 1% additive, SEM: standard error of the means

Dietary inclusion of additives particularly at 0.25% significantly increased ($P<0.05$) the eggshell thickness. This condition may be associated with the organic acid content in the feed additive, which increased the eggshell thickness and weight of the quail as previously reported by Fouladi *et al.* (2018). Indeed, the organic acid administration may improve mineral and protein absorption, which thereby improves the eggshell (Melaku *et al.*, 2021). It appears that the enhanced quality of eggshells may be linked to the positive impact of *S. cerevisiae* on calcium and magnesium metabolism, ultimately leading to improved eggshell formation. (Bidura *et al.*, 2019). Regarding the function of wheat bran as a

prebiotic, the role of such a component seemed to be minimal as Wanzenböck *et al.* (2018) did not find any significant effect of prebiotics on the eggshell quality. With regard particularly to feed conversion ratio, this measured parameter tended to be lower ($P=0.08$) in laying hens receiving additive at 0.25% of the diet.

Previous study has revealed that organic acids, prebiotics, and probiotics possess active compounds that are beneficial in improving the intestinal digestive and absorptive functions of chickens (Sugiharto *et al.*, 2021). Regarding this matter, the dietary inclusion of additives in this present study may be attributed to the improved intestinal functions and hence feed conversion of

hens. In the case of additives at the levels of 0.5 and 1.0% which did not have an impact on improving the feed conversion ratio, conditions that are too acidic in the intestines (due to the high content of organic acids in the feed additive) can lead to acidic stress that interferes with the function of the intestines (Nourmohammadi and Khosravinia, 2015).

The data on blood profile of laying hens are presented in Table 2. There is no significant ($P>0.05$) effect of the treatments on the most measured blood parameters, including leukocytes, erythrocytes, hemoglobin, hematocrits, thrombocytes, lymphocytes, heterophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Compared to the control, the dietary inclusion of additive

particularly at the level of 0.5% decreased ($P<0.05$) the levels of red blood cell distribution width-standard deviation (RDW-SD) and red blood cell distribution width-coefficient variation (RDW-CV) of laying hens during the post-peak egg production. A study that was conducted recently by Owoicho *et al.* (2022) suggested that high RDW values are associated with inflammatory conditions and infections. With regards to this matter, the reduced RDW-CV and RDW-SD in laying hens due to additive inclusion was attributed to fewer infections and inflammations. The antimicrobial properties of organic acids, prebiotics, and probiotics (Pratama *et al.*, 2022) may be responsible for the reduced pathogenic bacterial loads leading to fewer infections and inflammations.

Table 2. Complete blood counts of hens

Parameters	T0	T1	T2	T3	SEM	P value
Leukocytes ($10^9/L$)	125	97.9	88.9	104	7.65	0.40
Erythrocytes ($10^{12}/L$)	2.76	2.98	2.91	3.01	0.13	0.92
Haemoglobin (g/dL)	10.6	11.7	11.0	11.0	0.48	0.90
Hematocrits (%)	33.8	37.7	36.0	36.9	1.60	0.89
Thrombocytes ($10^9/L$)	15.2	9.80	9.40	11.8	0.96	0.11
Lymphocytes ($10^9/L$)	115	77.0	84.2	97.2	6.00	0.11
Heterophils ($10^9/L$)	10.0	6.90	6.80	7.20	0.83	0.50
MCV (fl)	123	126	125	124	0.62	0.51
MCH (pg)	38.5	39.2	37.7	36.7	0.47	0.30
MCHC (g/dL)	31.4	31.4	30.5	29.7	0.35	0.30
RDW-SD ($10^{-15} L$)	59.9 ^a	56.2 ^{ab}	50.2 ^b	54.6 ^{ab}	1.26	0.04
RDW-CV (%)	12.9 ^a	11.8 ^{ab}	10.6 ^b	11.7 ^{ab}	0.28	0.01
MPV ($10^{-15} L$)	8.76	8.96	5.50	8.96	0.14	0.52

^{a,b}In the same row, the means marked by superscript letters differ significantly ($P<0.05$)

T0: basal feed, T1: basal feed + 0.25% additive, T2: basal feed + 0.5% additive, T3: basal feed + 1% additive, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: red blood cell distribution width-standard deviation, RDW-CV: red blood cell distribution width-coefficient variation, MPV: mean platelet volume, SEM: standard error of the means

CONCLUSION

Novel feed additive containing *A. bilimbi* L. fruit filtrate, wheat bran, and *S. cerevisiae* particularly at 0.25% of diet improved hen day egg production (increased by 10.9%), eggshell thickness, and feed conversion ratio, without affecting the blood profiles of laying hens during post-peak egg production.

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