International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

The Use of Garlic (Allium sativum Linn) Bulb and Husk Powder as an Organic Feed Additive on Egg Quality of Laying Hens

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***Abstract*—** *This study was carried out to evaluate the egg quality of laying hens fed different levels of the garlic bulb and husk powder (GBHP). A total of three hundred and twenty, 30-weeks-old Isa Brown laying hens were randomly allocated into 8 dietary treatments with 4 replications (10 birds per replicate). The birds were fed either basal diet without feed additive (NC, negative control), basal diet supplemented with 0.015% virginiamycin antibiotic (PC, positive control), basal diet supplemented with garlic bulb and husk powder at the level of 0.25% (GBHP 0.25), 0.50% (GBHP 0.50), 0.75% (GBHP 0.75), 1.00% (GBHP 1.00), 1.25% (GBHP 1.25), or 1.50% (GBHP 1.50). The egg quality parameters observed were egg weight, eggshell weight, eggshell proportion, eggshell thickness, albumen weight, albumen proportion, Haugh units, yolk weight, yolk proportion, yolk color score, and yolk cholesterol content. Data were analyzed by using ANOVA. Duncan's multiple range test was used to determine the statistical significance among the treatment means. The results showed that the use of different levels of GBHP increased ($P<0.01$) egg weight, eggshell weight, eggshell proportion, eggshell thickness, and albumen weight. However, the diets had no effect ($P>0.05$) on albumen proportion, Haugh units, yolk weight, yolk proportion, yolk color score, and yolk cholesterol content. In conclusion, the inclusion of GBHP at 0.75% could be used as the organic feed additives to replace synthetic antibiotics without any negative effect on egg quality.*

***Keywords*—** *Egg quality, garlic, laying hens, organic feed additives.*

I. INTRODUCTION

Feed additives are the certain material which added or deliberately added to livestock diet in the small quantity. The addition of feed additives aims to increase the nutritional value of the diet to meet nutritional requirements, improve livestock health, productivity and products quality. Feed additives are also used to promote growth and increase the feed efficiency. Feed additives derived from plants have the potency to improve livestock performance and can produce livestock products which are environmentally friendly.

Feed additives which commonly used in the livestock diet are antibiotics from commercial or synthetic products which their safety aspect sometimes not guaranteed. The use of synthetic antibiotics in the diet is feared to cause resistance to pathogenic microorganisms which could endanger livestock health. Antibiotic residues in livestock products such as in the eggs could also threaten public health. These threats include changes in intestinal microflora of livestock which could trigger new diseases, as well as the threat of direct toxicity to humans who consume livestock products [1]. Based on the existence of these threats, the use of synthetic antibiotics as feed additives in Indonesia is currently banned, this is in accordance with the Ministry of Agriculture decree No.14/PERMENTAN/PK.350/5/2017 concerning the classification of veterinary drugs.

Organic feed additives have the potency to provide antioxidant effects, improve palatability, improve intestinal health, and increase growth and development of poultry so that they can produce well [2]. One source of organic feed additives to replace synthetic antibiotics is garlic (Allium sativum Linn). Garlic which contained allicin, essential oils, ajoene, and flavonoids was known to have strong antibacterial activity which helpful to maintain the birds health [3]. The use of garlic in the diet is aimed to improve poultry performance and health, so the poultry could produce more quality and safe products for human consumption.

Garlic which is processed both in the form of flour and juice could be used as a potential source of the organic feed additive. The inclusion of garlic juice to the level of 1.0% in laying hens diet could improve egg quality and reduced the amount of E. coli but did not affect the yolk cholesterol [4]. In another study, the use of garlic which prepared in the form of sun-dried paste could reduce the blood and egg yolk cholesterol [5]. Moreover, it was reported that garlic supplementation could affect egg production and yolk weight. Sun-dried garlic paste could be used up to 8% in the diet as a hypocholesterolemic agent. Supplementation of garlic flour with the level of 0, 5, and 10 g/kg diet could increase hen day production and egg weight, and decreased yolk cholesterol content, blood cholesterol, and triglycerides without causing adverse effects on laying hens [3]. Supplementation of oven-dried garlic flour to the level of 6% could act as a hypocholesterolemic agent which decreased the yolk cholesterol content without any significant changes on egg quality characteristics [6].

Garlic husk also contains flavonoids and organosulfur which act as antioxidants and antibacterials. The inclusion of garlic waste could reduce blood cholesterol in broiler [7]. Moreover, it was explained that the ability of garlic husk extract to reduce blood glucose levels may be due to the presence of several active compounds such as alkaloids, quinones, flavonoids, saponins, and polyphenols in the garlic husk. The highest concentration of flavonoids in garlic was found on the dry outer skin and the phytochemical content was higher than in the bulb, so the highest loss of flavonoids

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

occurs when the garlic was peeled [8], [9]. Therefore, this study was carried out to provide information about the use of garlic bulb and husk powder (GBHP) as a source of organic feed additive and its effect on egg quality of laying hens.

II. Materials and Methods

A. Birds and Diets

A total of three hundred and twenty, 30-weeks-old Isa Brown laying hens were used in this experiment. The birds had 3.37% of the coefficient of variation of egg mass. Each bird was placed into an individual battery cage. The diets used in this study were self-mixed diet and prepared in mash form. Nutrient content of the basal diet is shown in Table I. The calculation of the nutrient content of basal diet was based on the data from the Guideline of Hy-Line Brown Laying Hens Management, 2014. Feed additives used in this study were virginiamycin antibiotics and garlic bulb and husk powder. The diet was offered 125 g/bird/day, while the drinking water was offered *ad libitum*.

TABLE I. Nutrient content of basal diet.

Nutrient content	Calculated value
Metabolizable energy (Kcal/Kg)	2,853.60*
Dry matter (%)	88.78*
Ash (%)	11.04*
Crude protein (%)	17.74*
Ether extract (%)	5.43*
Crude fiber (%)	7.26*
Calcium (%)	4.34*
Total phosphorus (%)	0.78*
Available phosphorus (%)	0.49**
Lysine (%)	0.96**
Digestible lysine (%)	0.83**
Methionine (%)	0.44**
Digestible methionine (%)	0.41**
Natrium (%)	0.16**
Chloride (%)	0.16**

(*) Based on the analysis of Laboratory of Animal Feed, Department of Animal and Fisheries, Blitar District, 2018
(**) Based on the data of nutrient content from Guideline of Hy-Line Brown Laying Hens Management, 2014

B. Preparation of Garlic Bulb and Husk Powder

Garlic bulb and husk was sliced to small pieces and oven dried at 50^{o}C for 4 days. After that, the dried bulb and husk was finely ground to a powder at Laboratory of Materia Medica, Batu. The flavonoid content of GBHP was analyzed by using UV-Vis spectrophotometric method. The antioxidant capacity of GBHP was analyzed by using DPPH method, while the nutrient content of GBHP was analyzed by using proximate analysis. GBHP was ready to be included into the laying hens diet according to each treatment.

C. Experimental Design

The method in this study was *in vivo* experiment in a completely randomized design with 8 treatments and 4 replications. Each treatment consisted of 40 birds (10 birds per replicate). The treatments used in this study were basal diet without feed additive (NC, negative control), basal diet supplemented with 0.015% virginiamycin antibiotic (PC, positive control), basal diet supplemented with garlic bulb and husk powder at the level of 0.25% (GBHP 0.25), 0.50% (GBHP 0.50), 0.75% (GBHP 0.75), 1.00% (GBHP 1.00), 1.25% (GBHP 1.25), or 1.50% (GBHP 1.50).

D. Egg Quality Parameters

The egg quality parameters observed were egg weight (g/egg), eggshell weight (g/egg), eggshell proportion (%), eggshell thickness (mm), albumen weight (g/egg), albumen proportion (%), Haugh units, yolk weight (g/egg), yolk proportion (%), yolk color score, and yolk cholesterol content (mg/egg).

E. Statistical Analysis

The data of egg quality parameters were analyzed by using ANOVA. Duncan's multiple range test data was used to determine the statistical significance among the treatment means.

III. Results and Discussion

A. Characteristics of the Garlic Bulb and Husk Powder

This study began with laboratory analysis of GBHP samples which aimed to evaluate the nutrient content and bioactive compound (flavonoids and antioxidants) of GBHP. Results of proximate analysis showed that GBHP (based on 100% dry matter) had 89.27% of dry matter, 4.06% of ash, 16.11% of crude protein, 0.17% of ether extract, 6.68% of crude fiber, 0.27% of calcium, 0.40% of total phosphorus, and 4,242 kcal/kg of gross energy. GBHP contained low ether extract which was 0.17%. Low crude fat content in GBHP may be due to the evaporation of essential oils during the drying process. In this study, GBHP contained sufficient crude protein when compared to the crude protein content of garlic skin extract which was 0.57% [10]. The high crude protein content in GBHP was due to the combination of the garlic bulb and husk.

Results of the analysis of the bioactive compound showed that flavonoid and antioxidant contents of GBHP were 0.016% and 38.16%, respectively. This current study showed that even though the GBHP was heated to a temperature of 50oC, it still contained flavonoids and antioxidants which could be used in the laying hens. Flavonoids and antioxidants are part of organosulfur compounds which could act as antimicrobial or antibacterial, especially in improving the feed digestibility, which then could give effect on the quality of the eggs produced. Results of the bioactive compound analysis in this study were in contrary to the result of the previous study which stated that flavonoids content in garlic bulb powder could not be detected [11]. This contrary may be due to differences in the type of garlic used and the difference in sample preparation. The difference in the results of flavonoid content was also may be due to the sample used in this study consisted of the garlic bulb and husk. So that the detected flavonoids come from two parts of the garlic plant. The bioactive compounds contained in the garlic bulbs do not rule out the possibility that the flavonoids will also available on the garlic husk and have the same role as an antimicrobial agent [10].

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ISSN (Online): 2455-9024

B. Egg Quality of Laying Hens

Egg quality of Isa Brown laying hens in this study was divided into two parts namely external egg quality and internal egg quality. External egg quality of laying hens was shown in Table II, while internal egg quality was shown in Table III and Table IV.

External egg quality

Results of statistical analysis in Table II showed that the dietary treatments give highly significant effect (P<0.01) on the external egg quality includes egg weight, eggshell weight, eggshell proportion, and eggshell thickness. In this experiment, all GBHP treatments, except the GBHP 0.25, had higher egg weight compared to the NC treatment. This current finding was in accordance with the previous research which also reported that garlic juice supplementation up to 1% could increase egg weight of laying hens [4]. In another study, supplementation of garlic powder at the level of 5 and 10 grams had a highly significant effect on the increase in egg weight [3]. In contrary, [5] reported that egg weight was not affected by the use of dietary garlic paste which was supplemented at the level of 2, 6, 8 or 10% for 6 weeks of experimental period. These different results may be due to the differences in the use of the source of garlic, the preparation method, and the strain and age of the birds used.

TABLE II. Effect of the use of garlic bulb and husk powder on external egg quality of laying hens.

Treatment	Variable			
	Egg weight (g/egg)	Eggshell weight (g/egg)	Eggshell proportion (%)	Eggshell thickness (mm)
NC	57.75±0.78^{a}	7.06±0.14^{a}	12.24±0.19^{a}	0.337±0.003^{a}
PC	61.83±0.53^{c}	8.03±0.06^{bc}	13.00±0.14^{b}	0.361±0.002^{c}
GBHP 0.25	58.47±0.19^{a}	7.31±0.11^{a}	12.52±0.21^{a}	0.339±0.006^{a}
GBHP 0.50	59.58±0.25^{b}	7.81±0.11^{b}	13.12±0.18^{b}	0.348±0.003^{b}
GBHP 0.75	61.17±0.19^{c}	8.11±0.18^{c}	13.27±0.28^{b}	0.355±0.006^{bc}
GBHP 1.00	61.67±0.68^{c}	8.31±0.21^{c}	13.43±0.29^{b}	0.362±0.005^{c}
GBHP 1.25	60.14±0.29^{b}	7.94±0.06^{b}	13.23±0.15^{b}	0.354±0.005^{bc}
GBHP 1.50	59.72±0.74^{b}	7.83±0.14^{b}	13.12±0.36^{b}	0.351±0.005^{b}

$^{a-c}$ different superscripts within column showed a highly significant differences (P<0.01)
NC (negative control): no feed additive, PC (positive control): 0.015% virginiamycin antibiotic, GBHP 0.25: 0.25% garlic bulb and husk powder, GBHP 0.50: 0.50% garlic bulb and husk powder, GBHP 0.75: 0.75% garlic bulb and husk powder, GBHP 1.00: 1.00% garlic bulb and husk powder, GBHP 1.25: 1.25% garlic bulb and husk powder, GBHP 1.50: 1.50% garlic bulb and husk powder

Table II shows that diet containing GBHP, except the GBHP 0.25, had higher eggshell weight, eggshell proportion, and eggshell thickness compared to the NC treatment. The increased in eggshell weight and eggshell proportion were possibly because of the calcium and phosphorus content in GBHP. In addition, GBHP also contained flavonoids which could support in the process of nutrients absorption. Some factors that affect mineralization and eggshell quality were genetic, environment, nutrition and health of livestock [12]. In the previous study, it was reported that supplementation of herbal extracts, probiotics, and chitosan could increase calcium absorption, which then may possibly increase the eggshell quality of laying hens.

In this current finding, the increase in the eggshell thickness as affected by GBHP may be related to the increase in calcium retention from the digestive tract. In addition, the nutrient content and bioactive compounds of GBHP also contributed directly and indirectly in increasing the eggshell thickness. This increase in eggshell thickness was in line with the increase in egg weight, eggshell weight, and eggshell proportion. Bioactive compounds in the form of flavonoids and antioxidants in GBHP could effectively increase calcium absorption in the digestive tract so that it could increase the eggshell thickness.

In this current experiment, laying hens fed GBHP 0.75 and GBHP 1.00 diets had statistically equal egg weight, eggshell weight, eggshell proportion, and eggshell thickness compared to those fed PC diets. These results indicated that GBHP had a potency to be used as antibiotics alternative. The use of GBHP at the level of 0.75% seemed to be the most proper treatment due to the economic reason, as the increase in GBHP supplementation level may cause the increase in feed cost.

Internal egg quality

Albumen quality: The results of statistical analysis in Table III showed that dietary treatments had a highly significant effect (P<0.01) on albumen weight but did not give a significant effect (P>0.05) on albumen proportion and Haugh units. The increase in albumen weight was in line with the increase in GBHP supplementation level and increase in egg weight. The use of GBHP 0.75, GBHP 1.00, GBHP 1.25, and GBHP 1.50 diets had higher albumen weight compared to NC diet. In addition, those level of GBHP supplementation also resulted in the equal albumen weight compared to PC treatment.

The increase in albumen weight may be related to the increase in egg weight. The flavonoids content in GBHP was expected to be able to increase the nutrient absorption, particularly protein so that it could increase albumen weight. Protein will affect albumen synthesis. Protein content in the form of essential amino acids, particularly lysine and threonine, affects egg characteristics such as egg size, yolk, albumen deposition and internal egg quality [13].

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

TABLE III. Effect of the use of garlic bulb and husk powder on albumen quality of laying hens.

Treatment	Variable		
	Albumen weight (g/egg)	Albumen proportion (%)	Haugh units
NC	34.86±0.23[a]	60.36±0.54	81.96±1.69
PC	37.81±0.57[c]	61.09±0.49	82.84±3.94
GBHP 0.25	35.83±0.41[a]	61.24±0.56	83.22±0.67
GBHP 0.50	36.22±0.48[b]	60.76±0.63	82.82±1.29
GBHP 0.75	37.25±0.47[bc]	60.88±0.87	82.44±1.10
GBHP 1.00	37.61±0.74[c]	60.91±0.58	81.83±4.09
GBHP 1.25	36.50±0.58[bc]	60.64±1.01	83.17±2.93
GBHP 1.50	36.25±1.09[bc]	60.66±1.14	82.18±0.49

[a-c] different superscripts within column showed a highly significant differences (P<0.01)
NC (negative control): no feed additive, PC (positive control): 0.015% virginiamycin antibiotic, GBHP 0.25: 0.25% garlic bulb and husk powder, GBHP 0.50: 0.50% garlic bulb and husk powder, GBHP 0.75: 0.75% garlic bulb and husk powder, GBHP 1.00: 1.00% garlic bulb and husk powder, GBHP 1.25: 1.25% garlic bulb and husk powder, GBHP 1.50: 1.50% garlic bulb and husk powder

In this study, results of the Haugh unit of the eggs of Isa Brown laying hens were 82.56 ± 0.53. The value of Haugh units of laying hens fed diet containing GBHP had almost the same as those fed diet containing virginiamycin antibiotic (PC). This finding indicated that dietary GBHP in laying hens diet could be used to replace virginiamycin antibiotic in terms of improving the Haugh units. This result was in line with the research conducted by [3] which stated that garlic powder supplementation at the level of 5 and 10 g/kg of diet did not significantly affect the Haugh units. Haugh units is a value that reflects the state of albumen in order to determine egg quality. Egg quality can be measured based on albumen height, where the higher value of Haugh units, the higher albumen and this indicated that the eggs are still fresh. The Haugh units in this study was not in line with the increase in egg weight. Supplementation of GBHP in laying hens diet up to the level of 1.50% could improve the Haugh units of laying hens when observed from the average value obtained.

TABLE IV. Effect of the use of garlic bulb and husk powder on yolk quality of laying hens.

Treatment	Variable			
	Yolk weight (g/egg)	Yolk proportion (%)	Yolk color score	Yolk cholesterol content (mg/100g)
NC	15.08±0.31	26.14±0.34	7.33±0.13	242.92±2.29
PC	16.00±0.29	25.91±0.50	7.44±0.31	235.39±2.34
GBHP 0.25	15.33±0.24	26.24±0.44	7.72±0.21	233.79±3.17
GBHP 0.50	15.61±0.23	26.21±0.44	7.58±0.25	223.19±2.52
GBHP 0.75	15.81±0.48	25.82±0.70	7.64±0.21	215.70±2.48
GBHP 1.00	15.75±0.34	25.58±0.76	7.56±0.09	220.60±2.10
GBHP 1.25	15.69±0.65	26.14±1.02	7.61±0.06	217.97±4.06
GBHP 1.50	15.64±0.38	26.22±0.88	7.39±0.06	219.47±0.93

There were no significant differences among treatments (P>0.05)
NC (negative control): no feed additive, PC (positive control): 0.015% virginiamycin antibiotic, GBHP 0.25: 0.25% garlic bulb and husk powder, GBHP 0.50: 0.50% garlic bulb and husk powder, GBHP 0.75: 0.75% garlic bulb and husk powder, GBHP 1.00: 1.00% garlic bulb and husk powder, GBHP 1.25: 1.25% garlic bulb and husk powder, GBHP 1.50: 1.50% garlic bulb and husk powder

Yolk quality: Table IV shows the effect of GBHP on yolk quality of laying hens. The results of statistical analysis showed that the addition of GBHP in the diet had no significant effect (P>0.05) on yolk weight, yolk proportion, yolk color score, and yolk cholesterol content. The similar result of yolk weight was expected because of the fat content in the feed for egg yolk synthesis was relatively equal in all treatments. The average yolk weight which was affected by adding GBHP to the level of 1.50% was 15.61±0.36 g/egg, this result was relatively lower compared to the study of [3] with the supplementation of garlic flour as much as 5 and 10 g/kg diets could produce yolk weight of 17.68±0.25 g/egg. This may be due to the presence of flavonoids in the GBHP in the form of myricetin, quercetin, kaempferol, and apigenin which play a vital role in the fat synthesis so that the fat was not deposited into the yolks but was used as source of energy for production purpose. Addition of flavonoids in the form of quercetin could increase laying rate, eggshell strength, egg protein content and haugh units and reduce fat content in the yolks so that the yolk weight decreased [13].

The results of statistical analysis showed that the use of GBHP in laying hens diet up to 1.50% did not give a significant effect on yolk color score (Table IV). This finding may be due to the low content of the coloring agent or xanthophyl pigment in GBHP. In addition, it was also suspected that the pigment content in garlic was lost due to the drying process during the preparation of GBHP. The similar basal diet was also expected to cause the yolk color score was not significantly different.

The results of statistical analysis showed that the addition of GBHP in laying hens diet did not give a significant effect (P>0.05) on yolk cholesterol contents, however, the inclusion of GBHP numerically had lower yolk cholesterol contents compared to NC and PC treatments (Table IV). Dietary GBHP played a vital role in the nutrient digestion but had not been optimal in reducing yolk cholesterol contents. The decrease in

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yolk cholesterol contents may be due to the presence of several unanalysed bioactive compounds in GBHP which can reduce endogenous cholesterol synthesis in the liver, namely allicin and saponin, as previously observed by [3] who reported that unsaturated disulphide-oxide (allicin) could inhibit hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and cholesterol 7a-hydroxylase enzyme in the cholesterol biosynthesis. In another study, saponin was reported as potential agents not only to reduce yolk cholesterol contents but also to improve laying performance and egg quality [14]. Diosgenin (steroid saponin) was a very beneficial compound which could control hypercholesterolemia by inhibiting cholesterol absorption and increasing cholesterol excretion in feces [15].

The decrease in cholesterol level of yolk in this study was in line with the increase in supplementation level of GBHP. The increase in the level of GBHP supplementation caused yolk cholesterol content decreased. This research is in line with the previous study conducted by [5] who reported that the yolk cholesterol contents decreased significantly with the increasing levels of garlic extract. The addition of garlic extract with a level of 0.5, 1 and 2 g could reduce yolk cholesterol contents of laying hens [16]. Supplementation of garlic flour to the level of 3% could significantly reduce yolk cholesterol content and improved the ratio of yolk SFA and PUFA [17]. The inclusion of sun-dried garlic powder up to the level of 5% with Cu supplement could be used as hypocholesterolemic agent in laying hens diet without any significant effect on the laying performance and egg quality characteristics [18]. The use of oven-dried garlic to the level of 6% could act as a hypocholesterolemic agent in which the yolk cholesterol content decreased without any significant effect on egg quality characteristics [6]. The difference in the results of those mentioned study was may be caused by the differences in garlic used and preparation process, as well as the differences in age and laying hens strains.

IV. Conclusion

The inclusion of garlic bulb and husk powder in laying hens diet could be used as organic feed additive to improve both of external and internal egg quality, without any negative effect on laying hens. Garlic bulb and husk powder supplementation at the level of 0.75% could be used as an alternative of virginiamycin antibiotic to improve egg quality.

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