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Effect of Combination of Encapsulated Black Cincau Leaves (*Mesona Palustris* BL) and Probiotics on Production Performances, Yolk Cholesterol Content and Ammonia Level of Laying Hen

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ABSTRACT

The purpose of this research was to determine addition of natural feed additives from combination of encapsulated black cincau leaves and probiotics on feed intake, feed conversion, hen day production (HDP), egg mass, income over feed cost (IOFC), egg weight, yolk cholesterol content and ammonia levels in excreta. One hundred ninety-two laying hens at 28 weeks were used in this experiment. Egg mass which used before this research was 64.63±2.97 g/ day with CV was 4.59%. The method which used was experimental of completely randomized design (CRD) with four treatments and six replications (eight-layers each). The treatments used were T0: basal feed; T1: basal feed + combination of encapsulated black cincau leaves and probiotics 0.5%; T2: basal feed + combination of encapsulated black cincau leaves and probiotics 1%; T3: basal feed + combination of encapsulated black cincau leaves and probiotics 1.5%. Data were analyzed by using analysis of variance, if any significant effect, it would be further tested by Duncan's Multiple Range Test. The result showed that no siginficant effect (P>0.05) on feed intake, feed conversion, HDP, egg mass, IOFC, egg weight and yolk cholesterol content, but any significant effect (P< 0.05) on ammonia level. This research concludes that using 1.5% of combination of encapsulated black cincau leaves and probiotics give better result than others.

Key words: Black cincau leaves, Egg quality, Encapsulated probiotic, Hen production, Laying hen

INTRODUCTION

The term of probiotic mean life microorganism which have beneficial effect for its host. Probiotics are classified by the US Food and Drug Administration as generally recognized as safe (GRAS) ingredients (Patil et al., 2015). Commonly syntetic Antibiotics Growth Promoter (AGP) was used as growth promoter in poultry. AGP was used by farmer to increase egg production. Otherwise, using uncontroled AGP produce chemical residue on laying hen and its eggs, which can be harmful for human health. AGP was under supervised by many association, and have been removed from many countries (Ratcliff, 2000).

Developing sustainable livestock in poultry sector in the world should be considered as a good solution. Nowdays, the researchers are not only to focus on the enhancement of productivity, but also on food healthy and safety (Sienny and Serli, 2010). Especially in the developing country of Indonesia, this tropical country has high temperature and humidity. Those conditions could trigger heat stress on egg of laying hen (Li et al., 2015). Heat stress is able to disturb the health and reducing feed intake, then followed with decreasing productivity. Feed in poultry farm have highest production cost (approximately 70-80%). That all of the reason, feed should be efficiently used. Feed efficiency can be improved by adding feed additive (Teguie et al., 2004).

Using natural feed additives is needed. Natural feed additive might use probiotics and phytobiotics from herbal plants. Probiotics is single culture or mixture of living consumable microbes for human and/or animals and it has beneficial effect for its host (Patil et al., 2015). Its mechanism process is done in order to preserve the natural microflora balance within the body. Several benefit of addition of probiotics as feed for chicken would be decreasing mortality and cholesterol, increasing

hemoglobin (Hb) concentration, Packed Cell Volume (PCV), villus height also inhibit *Eschericia coli* and *Enterococci* (Rahman et al., 2013; Vantsawa et al., 2017; Bitterncourt et al., 2011; Pourakbari et al., 2016; Song et al., 2014). Cholesterol is a fat component composed of triglycerides, free fatty acids, and phospholipids. Eggs contain protein, carbohydrates, water, vitamins, mineral and cholesterol content, in one egg reaches 230,7 mg/ 100 g (USDA, 2018).

Black cincau is underbrush plant with height between 30-60 cm and mostly grown in 150-1800 m above sea level. Its leaves contain bioactive compound such as antioxidants, antibacterial, antimutagenic, hepatoprotective, antihypertensive and antidiabetic properties. Those bioactive compounds supposedly can maintain gastrointestinal tract and improve small intestinal microflora, performances and egg quality of laying hen (Hung and Gow, 2002).

Combination of black cincau leaves and probiotic has potentially given positive effect. This combination is expected to improve the ability of digestive system and also to improve the digestive enzyme activities, so that it could absorb feed nutrients for its production. Combination of phytobiotics and probiotics were characterized as hygroscopic. High vulnerable potency to beat heat condition, should protected by encapsulation technology. Microparticles should be water-insoluble to maintain their structural integrity in the food matrix and in the upper part of the GI tract that was needed to preserve the matrix content, benefit of these compounds during processing and storing, also to preserve probiotics viability (Chavarri et al., 2012). Encapsulation technology can be used as an alternative oral delivery to maintain the viability of probiotics, and allow it in controlling the release of viable cells into the host gut (in small intestine) so that it can be benefits (Cook et al., 2014).

Encapsulation is the process of coating one or more material to protect sensitive materials, in this case is feed components. It can reduce feed degradation in small intestine. According to Natsir et al. (2013), polymer that often used was Arabic gum, skim milk and whey protein which are able to prevent oxidation in feed additive, preserve the bacteria to stay alive during storage and better emulsion.

Based on that brief description, a study concerning a combination of encapsulated phytobiotic from black cincau leaves and probiotic were observed on this research. This research was conducted to know about production performance (feed intake, Hen Day Production (HDP), egg mass, feed conversion, Income Over Feed Cost (IOFC), egg weight, ammonia level) and yolk cholesterol content of laying hen.

MATERIALS AND METHODS

Materials and diets

One hundred ninty two 28-week-old strain Lohmann brown were used tin this experiment. Pre-experiment showed that the HDP was 84.57%, egg mass average was 64.63±2.97 g and variation coefficient was 4.59%. Birds then were housed in 24 group cages sized 64 cm ×35 cm ×30 cm (eight birds each) at 25-32 ^{o}c. The cages were equipped with lighting for 16 hours, feeder, drinker, egg container and digital scale (5 kg capacity), in order to measure feed residue and egg weight every day.

Black cincau leaves were obtained from Ponorogo Regency, East Java province. Probiotics used in this study were *Lactobacillus* sp 5.4 ×10^{7} cfu/ g and *Bacillus* sp 2.4 ×10^{8} cfu/ g. Encapsulation process was made using 20 g arabic gum, 5 g whey and 0.06 g butylated hydroxy toluene, 100 g of green black cinacu leaves were extracted with ethanol 70% and then mixed with mixer at 2500-3000 rpm for 15 minutes. Furthermore, the extraction was dried in modified microwave at 60^{o}C for 20 minutes. Composition of basal feed and nutrients content in proximate analysis were showed in table 1.

Experimental design

This research using four treatments and six replications (eight birds each). Feed was given as restricted feeding, 120 g/ hen/ day and drink as ad libitum for five weeks (35 days). Feeding frequency was done once a day, in the morning. Experimental feeding method was done by mixing basal feed with encapsulated of black cincau leaves-probiotics. The treatments were used as follows: T0 = basal feed; T1 = basal feed + combination of encapsulated black cincau and probiotics 0.5%, T2 = basal feed + combination of encapsulated black cincau and probiotics 1%; T3 = basal feed + combination of encapsulated black cincau and probiotics 1.5%. Data were analyzed by completely random design of ANOVA. If there was significant ($P<0.05$) then it is tested using LSD test (Steel and Torrie., 1980).

Ethical approval

This research did not involve the introduction of any intervention on birds. The data collcetion was obtained with humanly handled, which according of animal care and welfare standard of Republic Indonesia.

Table 1. Composition and nutrients content of basal feed[1]

Feedstuffs ingredients	Percentage (%)
Soybean meal	19.42
MBM	7.96
CGM	0.97
Maize	48.56
Rice bran	14.57
Premix	1.75
Binder	0.19
Salt	0.19
O-lalat	0.005
Orgacid	0.05
Vitamin C	0.01
Grit	6.31
Total	100
Analyzed feed composition	**Content**
Dry matter (%)	88.89
Ash (%)	13.87
Crude Fiber (%)	4.17
Crude Protein (%)	19.90
Crude Fat (%)	4.59
Gross Energy (MJ/ kg)	16.58
Metabolizable Energy (MJ/ kg)	11.61

[1]Proximate assay analyzed by Nutrition and Animal Feed Laboratory, Animal Science Faculty, Universitas Brawijaya, MBM= meat bone meal, CGM= corn gluten meal, MJ/ kg= megajoule per kilogram

RESULT AND DISCUSSION

Effect of combination of encapsulated black cincau leaves (*Mesona palustris* BL) and probiotics on production performances and yolk cholesterol content of laying hen have observed. The result in this research showed in Table 2.

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on feed intake and feed conversion

The result has shown in table 2. There is no significant effect (P<0.05) of combination of encapsulated black cincau leaves (*Mesona palustris* BL) and probiotics on feed intake and feed conversion. Feed intake in this research (T0, T1, T2 and T3) were reported 117.62 g, 118.34 g, 116.68 g, 118.05 g, consecutively. This result has been supposed to refer to the restricted feeding management which given once in a day. Furthermore, encapsulated black cincau leaves and probiotics have not influence on palatability, which respectable to feed intake. Factor affected feed intake were body size, genetic trait (breed), temperature, cage condition, feeder, condition of drinking water, quality and quantity of feed also the existence of disease. Oyedeji et al. (2005) reported that feed intake was influenced by feedstuffs and feeding method, such as feeding pigments. Moreover, different average value for each treatment would probably due to environmental factor.

There was significant difference (P<0.05) on feed conversion (Table 2). This might be supposed feed intake and egg mass in this research were also showed no significant difference (P>0.05). Feed conversion were reported 2.26; 2.28; 2.21; 2.11, consecutively. Several factor that influencing feed conversion were feed physical form, body weight, feed nutritional content, nursery environment, strain and sex. Another factors, such as temperature condition, damage of feed, feed quality and different location of housing have been reported to affected on feed conversion (Kelebemang, 2005; Suganthi et al., 2011; May and Lot, 2000).

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on HDP, egg mass, IOFC and egg weight

The result of HDP, egg mass, IOFC and egg weight have been shown in table 2. There was no significant difference (P>0.05) on those variables. HDP was reported, there was no significant difference (P>0.05) in this research. HDP result of this study were 84.57%, 84.64%, 83.00%, 89.80%, consecutively. It is supposed

due no significant difference (P>0.05) on feed intake in this study. Egg shaping of laying hen also depend on feed, which have been determined by its protein, fat, and calcium content. HDP is always related with egg shaping- production, thus egg production affected by feed intake and environmental condition. Reported from Awoniyi (2003) that different stage of housing influenced HDP of laying hen.

There was no significant effect (P>0.05) on egg mass in this research. Egg mass (g/ hen/ day) was reported as follow 53.24, 52.79, 53.72, 57.27, consecutively. This might occur because the addition of encapsulated of black cincau leaves and probiotic have not significant effect (P>0.05) on HDP. Measuring of egg mass (g/ hen/ day) was HDP × average egg's weight, otherwise increasing HDP will increase egg mass of this treatment. Protein and fat from feed can be digested as the constituent ingredient for both egg yolk and egg weight. Egg mass have also been influenced by the weight of egg yolk and egg whites. Numerically, treatment using 1.5% of combination of encapsulated black cincau leaves and probiotics could increase egg mass of laying hen. This might be due to active compound in treatment that is able to increase the digestion ability and improve digestive enzyme activities, so that it can absorb the feed and use it for digestion-metabolizing. Combination of phytobiotics-probiotics would balance the non-pathogenic bacteria population in digestive system, particularly those from genus *Bifidobacterium* and *Lactobacillus*.

There was no significant effect (P>0.05) of combination of black cincau leaves and probiotics IOFC. IOFC (Rupiah / hen/ day) in this research was showed 652.20, 546.75, 559.28, 600.85, consecutively. Higher result of IOFC was better than decreasing, because relate with economically analysis, such as income for farmer. Feed in laying egg farm have the highest production cost (60-70%) (Jahan et al., 2006). The best IOFC was obtained from basal feed (T0) with Rp (Rupiah) 652.20. This situation occurs because the combination of encapsulated black cincau leaves and probiotic has more expensive price (Rp 5,498/ kg – Rp 5,788/ kg) than basal feed (Rp 5,208/ kg).

There was no significant difference (P>0.05) on egg weight on this research. Egg weight (g) result showed 61.49, 62.44, 65.34, 64.51, consecutively. It was supposed that egg weight affected by several factors, either internal or external, such as genetic, environment, feed nutrients (including amino acids and mineral). This research used balanced-amino acid (methionine and lysin), with the same composition for each treatment. The best treatment was shown in T3 (using 1.5% phytobiotic-probiotic), which was supposed that amino acids content obtained from protein content of treatment.

Table 2. Average of feed intake, hen day production, egg mass, feed conversion, income over feed cost, egg weight, yolk cholesterol content and ammonia level in 28-week-old laying hen

Variables	Treatment			
	T0	T1	T2	T3
Feed intake (g/ hen/ day)	117,62±0.67	118.34±0.95	116.68±1.94	118.05±0.22
Feed conversion	2.26±0.21	2.28±0.19	2.21±0.10	2.11±0.17
HDP (%)	84.57±6.17	84.64±6.50	83.00±3.54	89.80±2.78
Egg Mass (g/ hen/day)	53.24±4.17	52.79±4.56	53.72±3.29	57.27±4.30
IOFC (Rp/ hen/day)	652.20±30.83	546.75±116.24	559.28±85.15	600.85±106.00
Egg weight (g)	61.49±3.59	62.44±1.44	65.34±2.51	64.51±2.18
Yolk cholesterol content (mg/ 100 g)	214.59±2.83	211.71±1.65	212.55±1.33	213.32±0.52
Ammonia level (ppm)	1.42±0.26^{b}	0.80±0.03^{a}	0.81±0.08^{a}	0.80±0.18^{a}

$^{a-b}$ means different superscripts (P<0.05); followed by different lowercase letters within a row are statistically different. HDP= Hen Day Production, IOFC = Income Over Feed Cost

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on yolk cholesterol content and ammonia level

The results showed that cholesterol levels of egg yolk (mg/100g) were 214.59, 211.71, 212.55, 213.32, consecutively. The result showed that there was significant effect (P≤ 0.05) on yolk cholesterol content. It is supposed that different metabolism condition of laying hen each, sample-drying method and several cholesterols distributed to meat. As numerically, T1 decreased cholesterol content on this research. This is because the content of antioxidants in treatment able in holding the cholesterol of egg yolk by inhibiting free radical formation of cholesterol. Flavonoids work in blood by

inhibiting enzymes 3-hydroxy 3-methylglutaril coenzyme a reductase (HMG CoA reductase), HMG CoA-reductase enzyme helps the formation of mevalonic acid and influencing cholesterol content (Nelson et al., 2000). Another studies showed that yolk cholesterol content carried out from serum cholesterol, i.e investigated by Kurtoglu et al. (2004) showed using 250 mg/ kg^{-1} probiotic either at 60^{th} or 90^{th} days were decreased.

There was significant difference ($P<0.05$) on ammonia level of laying's excreta. The result (ppm) was reported 1.42, 0.80, 0.81, 0.80, consecutively. It was supposed that ability of probiotic in this research to maintain small intestine of laying hen. Lactic acid bacteria express acid condition, which able to decrease ammonia gas when released. *Lactobacillus* sp. decrease the pH of excreta and number of gram-negative bacteria, it was because in acid environments would break-down metabolic processes in bacterial cells, such as metabolic formation of ATP (Adenosina Trifosfat). The condition is able to trigger the growth of non-pathogenic bacteria in the digestion tract and suppress growth of *Escherichia coli* and coliform, thus suppress production of hydrogen sulphide, which causes unpleasant-odor. Decreasing of urea content in the excreta was caused by the use of probiotics in poultry, also express an enzyme that works to hydrolyze urea into ammonia. Many studies showed that ammonia emission were decreased by probiotics, i.e *Lactobacillus salivarius* and *Pediococcus* pentosaceus on 12 d free chickens (Chen et al., 2017). Synergistic performance of encapsulated black cincau leaves and probiotics were seen able to trigger the increasing number of non-pathogenic bacteria in the intestinal villi. Otherwise, that the absorption of nutrient content occurs optimally, then the number of ammonia was decrease.

CONCLUSION

Using combination of encapsulated black cincau leaves and probiotics have not influenced on feed intake, HDP, egg mass, feed conversion, IOFC, egg weight and yolk cholesterol content, but decreased ammonia levels in excreta. Using 1.5% treatment gives best result then others.

DECLARATIONS

Consent to publish

Not applicable

Competing interests

The authors declare that there is no compete of interest in this research.

Author's contributions

The authors contributed to arrange the experimental research, determine method of research, preparation of materials method, research and data analyzed.

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