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EFFECT OF SINGLE AND MULTI-STRAIN PROBIOTICS ON EGG PRODUCTION AND BLOOD PARAMETERS OF LOCAL TEGAL DUCKS UNDER VILLAGE SYSTEM

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Abstract. This research aimed to study the effect of single and multi-strain probiotics on egg mass and blood status of local laying ducks under the village system. The method used was an experiment with a Completely Randomized Design. The treatment was single-strain probiotics and multi-strain probiotics with each level of 0/control, 3, 5, and 7 ml/kg feed. Each treatment was replicated 3 times, as a result, there were 21 flocks of duck. Each flock had 40 laying ducks, so there were 840 ducks involved. The treatment was applied for three months. The parameters observed were (1) egg mass; and (2) blood parameters. Results of this study indicated that egg mass was significantly higher (P<0.05) in the treatment group than that in the control group, and not significantly different (P>0.05) between the treatments. Erythrocyte, leucocyte, hemoglobin, and PCV count were not significantly different (P>0.05) between control and treatment, also among treatment groups. It can be concluded that the supplementation of probiotics either single or multi-strain has increased local Tegal duck egg production, however, this supplementation has no effect on blood parameters.

Keywords: ducks, single-strain probiotic, multi-strain probiotic, egg production, blood parameters.

Introduction

With a population of more than 58.6 million [1], ducks have a very strategic position for the Indonesian people The ducks provide not only good-quality food but also a large opportunity for villagers to gain income. Many people rely on this poultry for their main income, both as duck keepers and those involved in duck-related businesses. This position is strengthened by the fact that duck meat and eggs have a special place in Indonesian cuisine.

Tegal duck is one of the most famous local ducks which is an egg-producing type. In the past, most of the ducks were reared under an extensive scavenging system, but now most of them are kept under a semi-intensive management system. Under the system, the average egg production could be 237 eggs per year [2]. Improvement in local duck production is very important in terms of economic and nutritional points of view. Improvement in egg production could be done through several efforts and one of them is by supplementing feed with probiotics.

The Food and Agriculture Organization/World Health Organization (FAO/WHO) working committee on probiotics defined probiotics as “live microorganisms which when administered in adequate amounts confer health benefits on the host” [3]. Probiotics may be a potential alternative for improving gastrointestinal health and growth promotion in different animal species [4]. Probiotics are supplementary feeds in the form of microorganisms that can live in the digestive tract, are in symbiosis with existing microorganisms, are beneficial, and can increase growth and feed efficiency without undergoing the absorption process. Probiotics are products containing cell yeast, bacterial cultures, or both that are capable of stimulating microorganisms to modify the gastrointestinal environment to support health status and improve feed efficiency [5]. Probiotic mechanisms increase feed conversion efficiency including changes in the intestinal flora, increased growth of nonpathogenic, gram-positive, facultative anaerobes, formation of lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, improved digestion, and increased regulation of feed conversion efficiency [6]. Administrating probiotics could improve egg production, shell weight, and thickness of egg shells and reduce cholesterol levels in egg yolks [7].

The use of probiotics is considered favorable as a suitable option after the antibiotic ban. This is due to probiotic use for health has more benefits by resulting in safer products both poultry meat and eggs. The previous study indicated that probiotics could be used as antibiotic replacements for improving egg production of laying ducks [8].

Based on the microbial content, probiotics could be single-strain or multi-strain probiotics. A single-strain probiotic is defined as containing one strain of a certain species and consequently, multi-strain probiotics contain more than one strain of the same species or, at least of the same genus. Multispecies preparations have advantages compared to single-strain probiotics and, to a lesser extent, multi-strain probiotics [9]. Multi-strain probiotics are composed of more than one species or strains of bacteria and sometimes, include fungal species with benefits to human and animals’ health. While single-strain probiotics are beneficial to health, multi-strain probiotics might be more helpful because of synergy and additive effects among the individual isolates [10].

Egg mass which represents egg weight produced per duck was the most important trait in commercial egg-laying ducks since egg mass directly affected farm income [11]. In poultry, blood status is often used to evaluate the physiological status of birds. Therefore, information on blood status is one of the important things in an effort to increase production.

This research has been conducted to study the effect of single and multi-strain probiotics on egg mass and blood status of local laying ducks under the village system.

Materials and Method

This research was carried out directly on duck farms (on-farm) with the hope that the results obtained truly reflect the real conditions in the field and can be used directly by the farmers. This research collaborates with duck farmers who are members of the 'Berkah Abadi' Duck Farmers Group located in Pesurungan Lor Village, Margadana District, Tegal Town, Indonesia. The town and surrounding areas, which are home to local Tegal ducks, is one of the duck centers in the country.

The ducks were kept in sheds with rice straw bedding and solid floor ranch in front of the sheds. The sheds were located around farmers’ houses. All labor used was mainly family members which were not paid in cash. The use of supplementation feeds, and vitamins were rarely found. The vaccination program, mainly for New Castle disease and avian influenza was only conducted when local Livestock Service Offices provide the program. The average flock size is 245 ducks per farmer on average. Innovation in daily management is limited and economic evaluation has never been conducted. Most of the farmers used point-lay birds as replacement flocks instead of hatching eggs.

The research method used is an experimental design with a completely randomized design. Treatment is the provision of two types of probiotics namely single and multi-strain probiotics. Single-strain probiotic contains *Lactobacillus sp*., while multi-strain probiotic contains *Lactobacillus sp*. and *Saccharomyces cerevisiae*. Each probiotic had three levels i.e. 3, 5, 7 ml/kg feed, and a control group, therefore in total, there were 7 treatments. Each treatment was repeated 3 times, and it had 40 local ducks in the production period, so overall this study used 840 ducks. The data collection process was carried out for three months.

The probiotic treatments were given every morning by putting it in water to mix with the duck feed. The feed, which was given twice a day, was a mixture of bran, dried rice, and fresh fish (*pirik* fish or small *petek* fish) with a composition of 39.65%, 25.11%, and 35.24%, respectively. With this composition, the feed given has a nutritional value of 26.38% crude protein, 2,923 kcal/kg metabolic energy, 2.29% calcium, and 0.78% phosphorus. Every day ducks consume an average of 172 grams of feed. Drinking water was provided *ad libitum.*

Blood sampling for hematological observation was carried out at the end of the study, which was taken from four ducks (10%) in each cage plot and analyzed in duplicate. The variables measured in this study included: (1) duck productivity which was egg mass; and (2) blood parameters (erythrocyte, leucocyte, hemoglobin, and PCV). Some of the work procedures carried out are as follows:

1. Blood sampling procedure

Blood sampling for hematologic tests was carried out at the end of the study. Blood was taken from four ducks (10%) in each cage in duplicate, blood was taken from the wing vein (brachial vein), as much as 3 ml with a syringe then put in a sterile tube that already contained anticoagulant (EDTA/ Ethylene Diamine Tetraacetic Acid), labeled according to the duck number, the tube was inserted into a thermos and ready to be taken to the laboratory.

2. Hematologic examination procedures

a) The number of red blood cells or RBC (million/ml) was measured using a hemocytometer with the Hagem Dilution method [12].
b) The white blood cell count (WBC) was carried out using the blood smear method stained with Wright's reagent using Hematek Stain Pak according to the opinion of [13].
c) Hematocrit or PCV was determined with a Micro-capillary Reader [13].
d) The concentration of hemoglobin (g/100 ml) was measured by spectrophotometry [12].

3. Productivity measurement procedure:

The egg mass per duck is calculated by the number of eggs multiplied by the weight of the eggs divided by the number of ducks.

EM = (number of eggs x weight of eggs) : number of female ducks.

Data obtained was analyzed using variance analysis at a 5% level of confidence.

Results and Discussion

Average egg mass and blood parameters per treatment unit during this study are presented in Table 1

Table 1. Average egg production and blood parameters during the study

Parameters observed	Control	Single-strain Probiotic			Multi-strain Probiotic		
		3 ml	5 ml	7 ml	3 ml	5 ml	7 ml
Egg mass (g)	33.60 ± 4.25a	43.33 ± 3.74b	39.63 ± 4.39b	45.01 ± 3.97b	42.36 ± 3.20b	43.04 ± 3.62b	46.93 ± 4.16b
Erythrocyte (mil/µl)	2.94 ± 0.19a	3.18 ± 0.16a	2.85 ± 0.33a	3.21 ± 0.13a	2.97 ± 0.38a	3.07 ± 0.27a	2.60 ± 0.10a
Leucocyte (cells/µl)	8,875 ± 417a	8,017± 230a	7,933 ± 168a	8,042 ± 131a	9,467 ± 282a	7,917 ± 480a	8,980 ± 222a
Hemoglobin (g/dl)	8.4 ± 0.65a	8.8 ± 0.44a	8.4 ± 0.83a	8.4 ± 0.69a	8.8 ± 0.45a	8.8 ± 0.71a	7.9 ± 0.08a
PCV (%)	31 ± 4.3a	35 ± 3.5a	29 ± 1.7a	33 ± 1.5a	33 ± 1.5a	32 ± 3.5a	26 ± 1.0a

Note: a different superscript on the same line indicates a significant difference ($P<0.05$)

Egg mass

Table 1 shows that the egg mass varied from the lowest 33.60 grams in the control group to the highest 46.93 grams in the 7 ml multi-strain treatment. These figures were similar to previous research which reported that egg mass was 38 to 51 g [14], and between 36.07+3,25 to 45.27+4,57 g [8]. Based on statistical analysis, egg mass was significantly different ($P<0.05$) between the controls and the treatments, and not significantly different ($P>0.05$) between the treatments. Although it was not significantly different, it seems that supplementation of multi-strain probiotics resulted in higher egg mass than that of single-strain probiotics. It is most probably due to the improvement of feed efficiency under such probiotics. Probiotics also affect the anatomy of the intestine, both macroscopically and microscopically. Macroscopically, the chicken intestine becomes longer, and microscopically probiotics affect the density and length of the villi so that the surface area of the intestine for absorbing nutrients is wider in birds that receive probiotics. The long villi increase the absorptive capacity because the absorption surface area also increases. The wider the absorption surface, the better the ability of the intestinal mucosal cells to absorb nutrients [15]. Moreover, supplementing poultry feed with multi-strain probiotics was reported to result in a rise in villus length and the number of goblet cells in the jejunum, and villus height to crypt ratio in the ileum of chickens [16]. Multi-strain probiotics might be more helpful because of synergy and additive effects among the individual isolates [10]. This is presumably because multi-strain probiotics contain lactic acid bacteria and *Saccharomyces cerevisiae* so there are more types of microbial species. Several *Saccharomyces cerevisiae* strains have proven probiotic potential in both humans and animals [17]. Dietary *Saccharomyces cerevisiae* hydrolysate supplementation can stimulate the growth of broilers by regulating intestinal immunity and barrier function and improving intestinal morphology, which may be related to the enhancement of bacterial diversity and the changes in intestinal microbial composition [18]. *Saccharomyces cerevisiae* could serve as a growth enhancer by ensuring improved gut health which leads to improved digestive enzyme activities, growth performance, and nutrient digestibility. In addition, *Saccharomyces cerevisiae* and its derivatives could also serve as a possible alternative to in-feed sub-therapeutic antibiotics through their ability to modulate the immune response and also reduce the effect of pathogenic bacteria through mutual exclusion. This, in most cases, tends to result in improved bird performance and reduced mortality [19].

Blood parameters

In this study, the average total erythrocyte count was 3.02 + 0.26 mil/l, ranging from 2.55 ± 0.27 to 3.66 ± 0.62 mil/L. This result is in accordance with the results of another study which obtained the number of erythrocytes between 3.13 ± 0.11 up to 3.31 ± 0.10 million/L [20]. However, it is higher than the results of a previous study which reported the number of local duck blood erythrocytes during the production period of 2.3 ± 0.27 mil/L [21]. This is most likely due to differences in ducks and how they are managed. Hematological values in birds are influenced by age, geographical location, nutritional value, life habits of the species, and the present status of the individual [22]. Statistical analysis showed that the number of erythrocytes was not significantly different (P>0.05) between the control and treatment groups and between treatment groups. This shows that the supplementation of probiotics has no effect on the number of erythrocytes.

The overall average leukocyte count was 8,461 ± 632 cells/μl, with the lowest number being 6,200 ± 295 to 12,700 ± 376 cells/μl. This result is still lower than another study that found leukocyte counts of 10.550 cells//μl [23]. The method of rearing and age of the ducks could be contributed to these results. Based on statistical analysis, it turned out that there was no significant effect (P>0.05) from the probiotic treatment compared to the control and between treatments. It implies that the ducks in this study were in a healthy and safe condition, and no infection or mortality was found.

Hemoglobin from this study had an overall average value of 8.59 ± 0.62 g/dl with the lowest hemoglobin count being 7.65 ± 0.31 and the highest at 11.05 ± 0.45 g/dl. A study done on semi-intensive ducks indicated hemoglobin count was 8,03 ± 1,12 to 8,94 ± 0,84 [24]. Again, statistical analysis showed no significant effect (P >0.05) of the administration of probiotics on hemoglobin count, as well as single or multi-probiotics also had no significant effect (P>0.05).

The average PCV overall figure was 32 ± 3.9% with a range between 25 ± 1.8 to 40 ± 2.3%. These results are similar to previous studies, namely the average PCV was 36,857 ± 3,761 [21]. In terms of PCV treatment also had no significant effect (P>0.05). This proves that the addition of probiotics did not have a negative effect on the PCV bladder. The value of PCV in this study was 31.98 ± 3.9 % on average with the lowest of 25.0 ± 2.3 and the highest at 42.10 ± 3.2 %. The range of PCV values in this study was still in the range of local ducks, namely 37-44 [25]. Statistical analyses indicated that PCV values in control and treatment groups were not significantly different (P>0.05). Similar results have been reported in which research done on duck fed with fermented feed showed no significant effect on PVC value [26].

Conclusion

Supplementation of probiotics either single or multi-strains has increased local duck egg production, however, this supplementation has no effect on blood parameters.

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