

THE EFFECT OF *Lactobacillus salivarius* I-11 ON MICROBIAL POPULATIONS OF PELLET DUCK FEED

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ABSTRACT

An *in vitro* study was performed with the aim to evaluate addition *Lactobacillus salivarius* I-11 on population bacteria of pellet duck feed. The completely randomized design was been used on this research with 3 treatments and 3 replications with 5 experimental units. The treatments were P0 (Pellet feed duck without addition), P1 (Pellet feed duck with 2% addition *Lactobacillus salivarius* I-11) and P2 (Pellet feed duck with 4% addition *Lactobacillus salivarius* I-11). The parameters were total lactic acid bacteria, total bacteria, total fungi, and *Coliform* bacteria. The data obtained were analyzed variance (ANOVA) and further tests performed Duncan's Multiple Areas. The population of lactic acid bacteria was higher ($P < 0.05$) and the total bacteria, fungi and *Coliform* were lower ($P < 0.05$) with addition *Lactobacillus salivarius* I-11. The conclusion was addition of *Lactobacillus salivarius* I-11 as probiotics affects the lactic acid bacteria in pellet duck feed significantly ($p < 0.05$) increased but decreased the counts of total bacteria, total fungi and *Coliform*.

KeyWords : pellet feed, duck, *Lactobacillus salivarius*, population bacteria

INTRODUCTION

In intensive duck production, pellet feed are being applied that can affect to boost up productive performance. Adding probiotic as feed additives in pellet feed for ducks are alternatives antibiotics replacement. Probiotics are living microbes given orally to proliferate in the gastrointestinal tract (GIT) of the host and create beneficial conditions for nutrient utilization [1]

Giving probiotics provide beneficial effects such as a reduction in the ability of pathogenic microorganisms to produce toxins, stimulate the production of digestive enzymes and vitamins and antimicrobial substances that improve the health status of the host. Another advantage is the use of probiotics can reduce the negative pressure caused by the existence of barriers to the feed (such as anti-nutrients) on feed because probiotics are able to stimulate an increase in the availability of nutrients for the landlady [2].

Numerous studies have shown that specific strains of beneficial bacteria are able to reduce gastrointestinal pathogens such as *Salmonella* sp, *Colliform*, *E. Colli* and *Campylobacter jejumi* [3], [4]. *Lactobacillus* sp was the majority of probiotics that can give advantage for healthy such as : decreased cholesterol, increased gastrointestinal function and imunity [5]

Probiotics can be used in feed as an alternative of antibiotics to improve the growth of peking duck [6] and Shaoxing duck [7]. Lactic Acid Bacteria (LAB) probiotics can be used to improve feed efficiency and duck's performance [8]. The aim of the research was to evaluate microbiological quality of pellet duck feed with addition *Lactobacillus salivarius*.

MATERIALS AND METHODS

Materials

Research was done on Laboratory Feed Technology, Faculty of Animal and Agricultural Sciences, Diponegoro University. Materials research were *Lactobacillus salivarius* I-11 probiotics that isolated from ceca of Pengging duck [9]. Feed ingredients (corn mill, rice bran, fish meal, soybean meal, coconut oil, premix) mixing and pelleting. Ingredients and Chemical Composition of pellet duck feed which presented in Table 1.

Table 1. Ingredients and Chemical Composition of Diets

| Feed Ingredients | Composition (%) |
|---------------------------------|-----------------|
| Corn Mill | 55 |
| Rice bran | 19 |
| Fish Meal | 10 |
| Soybean meal | 15 |
| Coconut oil | 0.5 |
| Premiks* | 0.5 |
| Calculated chemical composition | |
| Energy (ME MJ/kg)** | 3115 |
| Crude Proteins (%)** | 20.8 |
| Crude Fat (%)** | 3.70 |
| Crude Fiber (%)** | 6.70 |
| Calcium (%)** | 0.6 |
| Phosphor (%)** | 0.8 |

*: Premix provided the following per kg of diet : Vitamin A-9500 IU. Vitamin E-30 mg, thiamine-1mg, pyridoxine-2 mg, folic acid-0.4 mg, riboflavin-6.5 mg, biotin-0.05 mg, Ca-panthotenate-10 mg, Fe-55 mg, choline chloride-350 mg, Se-0.2 mg, Zn-50 mg, Cu-5 mg and Mn-80 mg

** : AOAC (2000)

Microbials analysis

The microbial populations in the pellet duck feed (total lactic acid bacteria, total bacteria and total fungi) were analyzed by the method of [7]. The Most Probable Number (MPN) was used for determination of total *Coliform*.. Brilliant Green Lactose Bile Broth (BGLBB) as a cultured media used MPN method was incubated at at 37 °C for 24 – 48 hours. Afterwards, a confirmatory test was done using Lauryl Sulphate Tryptose Broth (LSTB) as a culture media which had incubated at 37 °C for 48 hours (SNI 2897,2008). There were two steps enrichment for qualitative *Salmonella* bacteria determination. Pre enrichment using Lactose Broth (LB) as a culture media had been incubated at 35 °C for 24 hours, followed by enrichment step using Selenite Cystine Broth (SCB) as a culture media that had been incubated at 43 °C for 24 hours. Isolation and identification method used Triple sugar Iron Agar (TSIA) and Lysine Iron Agar (LIA) that had been incubated at 35 °C for 24 hours.

Preparation of test sample

Pellet duck feed (1 g) was diluted in sterile diluents peptone water solution (9 ml) to make primary dilutions (10^{-1}). Then a series up to 10^{-5} dilution was prepared by transferring primary dilution (1 ml) into test tube containing test diluents (9 ml) to obtain 10^{-2} dilution and repeating the operations with sterile diluents (9 ml) using the 10^{-2} and further dilutions to obtain 10^{-3} , 10^{-4} , 10^{-5} 10^{-6} and/or 10^{-2} . The samples from each group were used

to enumerate the total bacteria, total fungi, total lactic acid bacteria and *Coliform* by selective culture medium.

One milliliter of appropriate serial dilutions of each sample was pour plate onto the sterile media. After 24 h incubation at 37 °C, the colonies that appeared on the plates were counted and the cfu ml⁻¹ was calculated.

Statistical Analysis

The parameters were populations of bacteria in the pellet duck feed (total bacteria, total fungi, total lactic acid bacteria and total *Coliform* bacteria) of duck. Experiment was conducted according to completely Randomized Design (CRD) consisted of 3 treatments and 3 replications with 5 experimental units. The treatments applied were P0 (Pellet feed duck without addition *Lactobacillus salivarius*), P1 (Pelet duck feed with 2% addition *Lactobacillus salivarius*) and P2 (Pelet duck feed with feed with 4% addition *Lactobacillus salivarius*). The data obtained were analyzed variance (ANOVA) to determine the influence of treatment and further tests performed Duncan's Multiple Areas to know the difference between treatments.

RESULTS AND DISCUSSION

Significant changes in microbial populations of pellet duck feed were found. The effect of *Lactobacillus salivarius* on population bacteria in pellet duck feed can be seen in Table 2.

Table 2. The Effect of *Lactobacillus* sp B12 on microbial populations of pellet duck feed

| Parameter | Perlakuan | | |
|-----------|-----------|----|----|
| | R0 | R1 | R2 |

| | -----cfu / gram ----- | | |
|----------------|-----------------------------|------------------------------|-----------------------------|
| Total Bacteria | 5.4 x 10 ^{7a} ±0.1 | 4.9 x 10 ^{6b} ±0.1 | 5.2 x 10 ^{6b} ±0.2 |
| Total LAB | 4.5 x 10 ^{1b} ±0.1 | 1.6 x 10 ^{6a} ± 0.2 | 2.4 x 10 ^{6a} ±0.1 |
| Total Fungi | 6.4 x 10 ^{6a} ±0.1 | 3.8 x 10 ^{2b} ± 0.2 | 1.5 x 10 ^{2b} ±0.1 |
| Colliform | 2.1 x 10 ^{3a} ±0.1 | 4.6 x 10 ^{1b} ±0.2 | 1.6 x 10 ^{1b} ±0.1 |

The statistical analysis showed that application of *Lactobacillus salivarius*. as probiotics affects the lactic acid bacteria in pellet duck feed significantly ($p < 0.05$) increased. The presented results are agreement with the findings of [7]. These changes in total lactic acid bacteria populations showed favourable effect of added probiotic on *Lactobacillus* concentrations in the ceca of duck. Addition *Lactobacillus agilis* and *Lactobacillus salivarius* enriched the diversity of *Lactobacillus* flora in the jejunum and ceca of chicken by increasing the abundance and prevalence of *Lactobacillus* inhabiting the intestine [10]. The predominance of lactic acid bacteria was related to the better performance of duck [11].

The counts of total bacteria and total fungi were decreased ($p < 0.05$) with probiotic treatments. The same effect showed with addition of antibiotic treatments decreased total microbe include fungi [12].

The effect of *Lactobacillus salivarius* on pathogenic bacteria in the ceca of ducks can be seen in Table 2 The statistical analysis showed that application of *Lactobacillus salivarius* as probiotics affects coliform bacteria in pellet duck feed significantly ($p < 0.05$) decreased. Similar findings have been reported by [13] Feeding lactic acid bacteria can decreased *Coli* counts and increase in gram-positive bacteria in the large intestine of broilers [14]. The reduction of pathogenic bacteria in the intestinal tract can be expected to improve nutrient digestibility and growth performance, alleviating weaning stress and lowering the inflammatory responses to sub-clinical infection [15].

Media with culture of *Lactobacillus salivarius* and *Lactobacillus plantarum* produce more acetic and lactic acid and the pH was lower [16]. *Lactobacillus salivarius* and *Lactobacillus plantarum* can ferment carbohydrates in poultry feed to produce pH levels and concentrations of lactic and acetic acid that inhibit the growth of pathogenic bacteria. The greater population on lactic acid bacteria with application *Lactobacilli* might inhibit harmful bacteria in the intestinal tract by blocking possible intestinal receptors of these pathogens or by secreting toxic metabolites against gram negative bacteria [13].

CONCLUSION

The conclusion was addition of *Lactobacillus salivarius* I-11 as probiotics affects the lactic acid bacteria in pellet duck feed significantly ($p < 0.05$) increased but decreased the counts of total bacteria, total fungi and *Coliform*.

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