EFFECTS OF PROBIOTIC SUPPLEMENTATION TO DIETS ON BLOOD UREA NITROGEN AND PLASMA CREATININE IN BROILER CHICKEN

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Abstract

This study investigated the effect of probiotic supplementation into diets on blood urea nitrogen (BUN) and plasma creatinine of broiler chickens. The probiotic used in this study is a commercial probiotic powder containing 3 dominant strains, namely Candida ethanolica, Monascus fumeus, *and* Bacillus subtilis*. One hundred broiler chickens were randomly assigned to four treatments. The treatments were the percentage of probiotic supplementation on basal diet: PP0 (control/basal diet with no probiotic supplementation; PP025 (basal diet with 0.25% probiotic); PP050 (basal diet with 0.50% probiotic); PP075 (basal dietwith0.75% probiotic). The probiotic powder in respective treatment was calculated based on amount of basal diet supplementation. The calculated amount of probiotic then mixed to the basal diet prior to supplied to the animals. The results showed that there was no significant difference in blood urea nitrogen (BUN) and plasma creatinine in any probiotic-fed birds compared to control group. However, there is a tendency of decrease in creatinine level in PP050, suggesting a lower rate of muscle expansion and kidneys function, although the difference was not significant. BUN and plasma creatinine were associated with the functionality and health of kidneys. In conclusion, the probiotic supplementation in the diet give neither benefit nor damage to renal health and functionality of broiler chickens.*

Key words: Probiotic powder, broiler chicken, plasma blood urea nitrogen, plasma creatinine

INTRODUCTION

Poultry production and industry has been considered as an important sector to provide food supply in most parts of the world. Therefore, the intensification of poultry production is accelerated significantly in last decade. However, intensive poultry production nowadays has negative impact on birds' health and welfare. The high-productive feature of broiler chickens has a trade-off: lower immune system that made them prone to diseases. Besides, intensive poultry production is often associated with stressful, disease-generating, and deteriorating environmental conditions that lead to more economic losses.

To overcome this trade-off, most of poultry farmers utilize the veterinary medicines to prevent and cure the health and productivity problems, that leads to substantial increase of veterinary medicine utilizations, especially antibiotics. However, the utilization of antimicrobial agents as a preventive measure has been questioned, in relation with the increasing number in evolutions of antimicrobial resistance of pathogenic bacteria. Thus, the search of alternative to antibiotic utilization has been emerging lately. One of the alternatives is the probiotics utilization [1].

Probiotic is defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [2,3]. The benefits that provided by probiotic supplementation were varied and acted in a wide aspect in nutrition and health, including enzymatic activity enhancing absorption of nutrients [4], ruminal digestion [5], fiber degradation [6], immunomodulatory effects [7], and regulation of intestinal microflora homeostasis, thus stabilizing mucosal barriers [8]. It seems that probiotic supplementation is

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promising for improvement of health and performance of animals.

One of the nutritional benefits of probiotic supplementation was the intestinal enzymatic activity that occurred in the gastrointestinal tract. In ruminants, it is already known that ruminal microflora [9] and enzymatic activity [10] influences the digestibility of feed supplemented to animals. In monogastric animals, such as mice, it was reported that *Bifidobacterium*-based probiotic supplementation enhanced gut microflora, thus reduced risk of obesity and improve its health status [11,12].

The study of effect of probiotic supplementation to plasma metabolites, especially in broiler chicken is lacking. Therefore, in relation with the nutritional benefit of probiotic supplementation, we considered that it might be important to study the effect of probiotic supplementation to the blood urea nitrogen (BUN) and plasma creatinine. BUN is known to be associated with renal function [13], while plasma creatinine level is associated with the muscle tissue mass metabolism rate and also renal function [14,15]. Based on previous literature evidences, we hypothesized that the higher probiotic supplementation on the diet of broiler chicken will result in normal BUN and plasma creatinine levels.

MATERIAL AND METHODS

Animals, housing, and experimental design

A total of 100 unsexed broiler day old chicks (CV. Missouri, Bandung, Indonesia) were used in this study. Each 5 birds were randomly housed in 1 of 20 pens (size: 0.7 m^2 per pen) that made from bamboo as partitions, and wood chips as bedding. The pens were attached with a feed and water bank on the front edge, in which animals were having access to calculated feed supply and ad libitum water. Prior to the experiment, all parts of housing were fumigated and washed with a detergent to prevent any bacterial contamination.

The experiment was conducted as a completely randomized design (CRD) with 4 treatments, namely PP0: basal diet with no probiotic supplementation (control); PP025: basal diet + 0.25% probiotic; PP050: basal diet + 0.50% probiotic; PP075: basal diet + 0.75% probiotic. Each 5 pens were randomly allocated into 1 of 4 treatment groups mentioned above, resulting 5 replications per treatment. The composition basal diet used in this study is depicted in Table 1, whereas the nutrient contents in every treatment is depicted in Table 2.

Table 1 Ration ingredients, their nutrient content (% as DM), and their metabolizable energy (kcal/kg)

Feedstuff	Nutrient Fraction[1]							
	CP (%)	CF (%)	CFib (%)	Ca (%)	P (%)	Met (%)	Lys (%)	ME (kcal/kg)
Corn	8.6	3.8	2,2	0.02	0.1	0.18	0.2	3,370
Probiotic	9.7	76	18.7	0	0	0	0	2,346
Soybean meal	44.0	0,9	6.0	0.32	0.29	0.65	2.9	2,240
Rice bran	12.0	12.0	3.0	0.04	0.16	0.27	0.71	2,860
Fish meal	58.0	9.0	1.0	7.70	3.9	1.80	6.5	2,970
$CaCO_3$	0	0	0	40.0	0	0	0	0
Palm oil	0	100	0	0	0	0	0	8,600
Amino acid additive	0	0	0	0	0	0.3	0.3	0
Lysine	0	0	0	0	0	0	80.0	0

[1]CP: Crude Protein; CF: Crude Fat; CFib: Crude Fiber; P: Phosphorus; Met: Methionine; Lys: Lysine; ME: metabolizable energy

Table 2 Nutrient contents in each treatment in this study (expressed in %, otherwise specifically stated)

Nutrient[1]	Treatment[1]			
	PP0	PP025	PP050	PP075
Crude Protein	20.64	20.53	20.43	20.33
Crude Fat	5.10	5.12	5.14	5.16
Crude Fiber	3.12	3.15	3.19	3.23
Ca	1.00	0.99	0.99	0.99
P	0.35	0.35	0.35	0.35
Lysine	1.17	1.16	1.15	1.15
Methionine	0.38	0.38	0.38	0.38
ME (kcal/kg)	3,070	3,071	3,071	3,071

[1]PP0: basal diet with no probiotic supplementation (control);
PP025: basal diet + 0.25% probiotic;
PP050: basal diet + 0.50% probiotic;
PP075: basal diet + 0.75% probiotic

Probiotic used in this study is a commercial probiotic product (Heryaki Powder, Bandung, Indonesia) that consist of three dominant bacteria such as *Candida ethanolica, Monascus fumeus,* and *Bacilus subtilis*. All bacteria colony was cultured in rice bran-based medium, to support most efficient bacterial growth.

Plasma metabolites determination

The blood samples were collected from one bird in each pen (20 birds in total), and placed in EDTA Vacutainer tube (BD Vacutainer, Plymouth, UK), prior to centrifugation at 3000 rpm for 15 minutes to isolate the plasma and stored in -30°C freezer till analysis. Blood urea nitrogen and plasma creatinine were determined by using the Urea Colorimetric Method Quantification Kit (Lot Nr. 80221, Biolabo, Maizy, France), read at 600 nm and Creatinine Kinetic Method Quantification Kit (Lot Nr. 80107, Biolabo, Maizy, France), read at 490 nm, respectively.

Statistical analyses

To determine the effect of treatment, a one-way ANOVA was performed by using proc GLM in SAS Statistics version 9.4 (SAS Institute, Cary, NC, USA). The level of significance used was $P<0.05$. The multiple comparisons of Duncan's Multiple Range Test were performed to compare treatments in every parameter. The data showed as least square means with pooled standard error of means across treatments.

RESULTS AND DISCUSSION

In the present study, no significant different found in all treatments to either levels of BUN or plasma creatinine ($p>0.05$). However, we found a numerically decrease of plasma creatinine levels in PP050 group compared to control group (22.4 vs 55.9 µmol/L). This finding is similar to previous study in ostrich chicken, which a decrease of creatinine was found in the plasma that fed *Bacillus subtilis*-based probiotics [16]. Although no significant different found, numerically lower plasma creatinine levels tended to indicate that there is lower muscle metabolism rate in PP050 group compared to control, suggesting a lower rate of muscle tissue mass expansion [14]. Except for PP050 group, creatinine level across all treatments in this study is lie within the normal level (40 to 133 µmol/L) [15,17].

Table 3 Effects of probiotic supplementation to blood urea nitrogen (BUN; mg/dL) and plasma creatinine (µmol/L) of broiler chickens

Variable	Treatment[1]				SEM[2]	*p*-value	Sig.[3]
	PP0	PP025	PP050	PP075			
Blood Urea Nitrogen (mg/dL)	66.4	68.5	56.5	58.1	14.2	0.9	NS
Creatinine (µmol/L)	55.9	41.0	22.4	32.6	11.1	0.1	NS

[1]P0: basal diet with no probiotic supplementation (control);
P1: basal diet + 0.25% probiotic;
P2: basal diet + 0.50% probiotic;
P3: basal diet + 0.75% probiotic.
[2]Pooled standard error of means
[3]NS: not significant

In this study, we used commercial probiotic contains three dominant strain of bacteria, namely *Candida ethanolica*, *Monascus fumeus*, and Bacillus subtilis. In a recent study, *C. ethanolica* strain showed the best results on NDF digestibility during the fermentation and showed better results than the control treatment at all fermentation times [18]. The results of that study suggest that *C. ethanolica* is one of the best strains to be utilized as ruminal probiotics. Meanwhile, *Monascus sp.* produces dimumeric acid, γ-aminobutyric acid (GABA) [19], and monacolin K [20]. Monacolin K has the functions as an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis. It is also known as mevinolin, lovastatin, or mevacor, and used as an antihypertension agent [21]. However, there is a lacking in literature regarding the efficacy of *Monascus fumeus* as probiotics, since this strain is just recently identified and characterized [19].

On the other hand, *B. subtilis* is an extensively studied bacterial strain. *B. subtilis* is a gram-positive bacteria that already used as an alternative medicine as immunomodulatory agent [22, 23] and enzyme-producing agent [24]. *B. subtilis* have become a one of the most popular strains due to their superb fermentation properties, high production rate (20 to 25 gram per liter), and the complete lack of toxic by-products [24]. Moreover, featured with high-yield protein secretion characteristics, *B. subtilis* is regarded as one of the key drivers in enzyme, probiotics, and protein production industry [24].

CONCLUSIONS

Based on the results of present study, no significant difference found in the probiotic supplemented group. However, a tendency of decrease in plasma creatinine level in PP050 suggesting a lower muscle tissue mass metabolism rate in this group. Apart from that, the results suggested that the probiotic supplementation in the diet give neither benefit nor damage to renal health and functionality of broiler chickens. However, the potential benefit of *Candida ethanolica*, *Monascus fumeus*, and *Bacilus subtilis*-based probiotic is promising, in relation with the increasing awareness in bacterial culture and protein secretion developments.

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