



The Effect of Dried Probiotics on Broiler Performance and Kidney Function

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Abstract | This research to assesses the dried probiotic impact on broilers health as measured by uric acid, blood urea nitrogen, and blood creatinine. The study was conducted at Test Farm Animal Husbandry Faculty, Universitas Padjadjaran, from 6 August to 6 September 2019. A total of 100 day-old-chick were randomly assigned to five treatments and four replications using Completely Randomized Design. Dried probiotics used cow's milk, soybean milk, and mung bean milk in combination with the oven-dry method. Dried probiotics can protect kidney cells from stress and free radicals in late-phase laying hens, which can be demonstrated by normal levels of creatinine, uric acid, and urea, thereby increasing productivity. The treatment consists T0: basal rations, T1: basal rations + 100% CM dried probiotic, T2: basal rations + 75% CM + 25% fermented MBM dried probiotic, T3: basal rations + 75% CM + 25% SM dried probiotic, and T4: basal ration + 50% CM + 25% MBM + 25% SM. Each treatment was given as much as 2% dried probiotic of the total ration. The results showed that T3 had the lowest ($P > 0.05$) FCR of all treatments. The average body weight in T2 treatment was greater ($P < 0.05$) than other treatments. Supplementation of dried probiotics in T2 increased carcass percentage ($P > 0.05$) compared to other treatments. The average blood creatinine level in T0 was lower ($P < 0.05$) than other treatments. Blood urea nitrogen level in T0 was lower ($P > 0.05$) than other treatments. Whereas for uric acid level, T3 was lower ($P < 0.05$) than other treatments. Supplementation of dried probiotics can improve growth performance and provide health to kidney function.

Keywords | Dried probiotic, Broiler chicken, Uric acid, Kreatinin, Blood urea nitrogen, Kidney function

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INTRODUCTION

Broiler chicken meat is a relatively affordable source of animal protein, which is vital in meeting nutritional needs. Rapid growth in broiler chickens is always followed by high lipid levels, which becomes a problem for people with degenerative diseases. Therefore, modification in feed is needed to reduce lipid levels in broiler chicken meat. It is also derived from broiler chickens with healthy kidneys. Kidneys are involved in a variety of vital processes. Consequently, it is not surprising that kidney dysfunction

can result in significant disadvantages for the broiler industry (Sultana *et al.*, 2021). Kidneys are organs responsible for filtering and excreting metabolites that the body no longer needs. The kidney's role is to filter blood and concentrate metabolic waste into urine (Scott and Quaggin, 2015). The residues of metabolism are uric acid, creatinine, and urea in the blood. These three metabolites are also indicators of kidney health and function. Any abnormal increase in serum levels of uric acid and creatinine may imply kidney damage (Yalcin *et al.*, 2012).

Creatinine is a substance produced constantly by muscle metabolism. An increase in creatinine levels can indicate kidney damage. Creatinine is produced and excreted in proportion to muscle mass. Most of the protein metabolism is transported in the blood before excreted from the kidneys in the form of uric acid. Uric acid levels in the blood can illustrate the results of body protein metabolism (Elizabeth, 2009). Blood urea is the concentration of urea in plasma, which amount depends on amino acid catabolism levels in the liver. From the liver, urea distributes to the kidneys to be filtered and excreted in the urine (Murray *et al.*, 2009).

Modification of feed in broiler chickens can minimize kidney damage. Probiotics can increase ration efficiency and body weight; reduce cholesterol levels in blood and meat, non-protein nitrogen in the blood, the concentration of uric acid, ammonia, and urea in the blood (Kumalasari *et al.*, 2020) Probiotics are live microbes that are beneficial for the health of the digestive tract because they provide a balance to the microbial ecosystem. The role of probiotics allows the digestibility and absorption of nutrients to increase by suppressing pathogenic bacteria in the digestive tract to support the development of good bacteria that help the absorption of nutrients (Adriani *et al.*, 2019).

The probiotic media in this study included cow's, mung bean, and soybean milk. These nuts contain isoflavone aglycone active substances, which can affect the levels of creatinine, blood urea, and uric acid. Flavonoids can help increase blood flow to the kidneys. The probiotic microbes used consist of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*. *Lactobacillus acidophilus* and *Bifidobacterium spp.* which has better resistance in the human digestive tract (Adriani *et al.*, 2019). This study examined the effects of dried probiotics made from cow's, mung bean, and soybean milk on broiler chicken performance and blood urea nitrogen, creatinine, and uric acid levels.

MATERIALS AND METHODS

MATERIALS

Soybean and mung bean (CV. Kurnia, Indonesia), cow's milk (KPSBU Lembang, Indonesia), probiotic starter of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, *Bifidobacterium bifidum* (Yogourmet, Canada), maltodextrin DE 10-12 (CV. Subur Kimia Jaya, Indonesia), creatinine reagen, uric acid reagen, and blood urea nitrogen reagen (Biolabo, Indonesia).

BIRD AND DIET

One hundred day-old-chick broilers with a body weight variation of 4.05% were used in the experiment. There were

five treatments and four replications. T-0= control; T-1= 100% fermented cow's milk; T-2= 75% fermented cow's milk + 25% mung bean milk combination; T-3 = 75% fermented cow's milk + 25% soybean milk combination; and T-4= 50% fermented cow's milk + 25% mung bean milk + 25% soybean milk. The feed has 21.37% protein and 3,066 kcal/kg metabolic energy (Table 1). Dried probiotics are given as much as 2% of the total ration.

Table 1: Nutrient content in feed.

Items	% as fed
Corn	57.30
Rice bran	4.50
Soybean meal	27.00
Fish meal	7.60
Coconut oil	2.00
Meat bone meal	1.50
Methionine	0.10
Total	100
Chemical analyze (% as-fed)	
Metabolizable energy (kcal/kg)	3070
Crude protein (%)	21.40
Crude fiber (%)	4.62
Crude fat (%)	1.49
Lysine (%)	1.29
Methionine (%)	0.51
Methionine + cystine (%)	0.65
Calcium (%)	0.98
Phospor (%)	0.66

DRIED PROBIOTIC

Soybean and mung bean milk are made by mixing cow's milk with beans. Cow's milk, soybean, mung bean milk, and the combination of it were fermented with *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* with 1:1:1:1 microbes ratio. Fermented milk was coated with maltodextrin 5% v/v before being dried using the simple dry oven at 40°C for 25 hours. After the drying process, the total number of lactic acid bacteria was $7,09 \times 10^2$ to $4,51 \times 10^3$. Although the total lactic acid bacteria is still below the Indonesian National Standard 2009 minimum standard, 10^7 CFU/ml, there is a high potential because mung beans and soybeans contain isoflavones in the form of aglycones, vitamin C, and vitamin E. This substance serves as an antioxidant, which influences growth and health (Kumalasari *et al.*, 2020a).

STUDY AREA

The study was conducted in a semi-closed house at the Test Farm Faculty of Animal Science Universitas Padjadjaran

Indonesia, from August to September 2019. Samples evaluation and probiotic powder creation in Poultry Production Laboratory Faculty of Animal Husbandry Universitas Padjadjaran, Indonesia.

DATA DETECTION

The formula for calculating feed conversion is feed consumption for a week divided by the amount of body weight gained for the same week. On the 28th day, samples were taken to measure carcass weight and carcass percentage from 20 experimental animals. Before slaughtering, data on slaughter weight was gathered by weighing live chickens individually following a 12-hour fast. After the chicken had been slaughtered, the carcass percentage was calculated, excluding blood and feathers.

Blood samples were taken from the pectoralis vein using a syringe inserted into the wing vein (vena pectoralis externa) as much as 3 mL. Centrifugation at 3000 rpm for 10 minutes was carried out at the Laboratory of Physiology and Biochemistry Nutrition Faculty of Animal Husbandry Universitas Padjadjaran. Serum was collected using a micropipette into the eppendorf tube to be stored at 4°C temperature until further examination (Rahmania et al., 2021). Blood urea nitrogen, serum creatinine, and uric acid levels were determined using standard reagent kits and analyzed using a semi-automatic chemistry analyzer.

STATISTICAL ANALYSIS

Data were collated and analyzed using SPSS ver. 13.00 with one-way analysis of variance (ANOVA). Duncan’s test was used to identify significant treatment mean differences at p<0.05.

RESULTS AND DISCUSSIONS

Table 2 shows data on the supplementation of dry probiotics on feed conversion ratio (FCR), body weight, and carcass percentage of experimental broiler chickens.

FEED CONVERSION RATIO (FCR)

Feed Conversion Ratio (FCR) is the ratio between

the amount of ration consumed and the resulting body weight gain. The average FCR of broiler chickens ranged from 2.05 to 2.62. The highest FCR was T0, 2.62 ± 22.77 and the lowest was T3, 2.05 ± 11.88. Based on the results of the Duncan test, dried probiotics supplementation significantly (P< 0.05) decreased the FCR. The decrease in the ration conversion rate from each treatment is respectively 13.35% (T1), 19.08% (T2), 21.75% (T3), and 16.79% (T4). T3 showed the lowest FCR (P <0.05) compared to other treatments, except with T2. T3 and T2 with a combination of fermented beans provides a good balance so that optimum aglycone activity can increase the efficiency of ration use. The optimum aglycone activity in dried probiotics causes the metabolism of LAB, which affects the absorption of nutrients in the digestive tract. In this circumstance, protein becomes optimum and digests smoothly (Kumalasari et al., 2020a). Probiotic improves nutrient digestibility so that it is efficient in increasing body weight without increasing the amount of feed intake (Lee et al., 2010; Zhang et al., 2011). This was shown by morphometric ileum and jejunum villi in the treatment with dried probiotics, which was higher than the control treatment, especially in T2 and T4. The height and width of the villi at T2 were 636.21 µm and 162.25 µm. Whereas in T4, where the height and width of the villi were 665.98 µm and 151.9 µm. The increase in height and width of the villi correlates with the absorption surface area of nutrients into the bloodstream. Probiotic in the ration increases enzymatic activity and digestive activity, so the nutrients usually wasted in the feces will be reduced.

Probiotics increase the feed conversion ratio (FCR), the small intestine’s growth, and the broiler villi height (Mohammadigheisar et al., 2019; Adriani et al., 2019). Moreover, adding probiotics to chicken feed can selectively expand the healthy bacterial community, promoting the growth and performance of chickens (Neijat et al., 2019). FCR is usually used as a benchmark for the maintenance of broiler chickens. In this study, dried probiotics supplementation in the ration showed a better FCR value, although it was still far from the FCR value achieved by commercial broiler farms.

Table 2: The effect of dried probiotics on performance broiler chicken.

Parameters	Treatments				
	T0	T1	T2	T3	T4
Feed conversion ratio	2.62 ± 22.77 ^d	2.27 ± 13.25 ^{cd}	2.12 ± 11.14 ^{ab}	2.05 ± 11.88 ^a	2.18 ± 3.72 ^{bc}
Body weight (g)	958.75 ± 16.96 ^a	1205.00 ± 5.50 ^{ab}	1286.25 ± 20.93 ^d	1240.00 ± 15.48 ^{bc}	1261.25 ± 12.54 ^{cd}
Carcass percentage (%)	72.01 ± 4.71 ^a	75.26 ± 1.02 ^{ab}	77.07 ± 5.01 ^d	76.83 ± 1.86 ^{bc}	77.06 ± 1.91 ^{cd}

Note: T-0 = control; T-1 = 100% fermented cow’s milk; T-2 = 75% fermented cow’s milk + 25% mung bean milk combination; T-3 = 75% fermented cow’s milk + 25% soybean milk combination; and T-4 = 50% fermented cow’s milk + 25% mung bean milk + 25% soybean milk. Duncan’s test indicates significantly different (P<0.05) letters in the same column.

Table 3: The effect of dried probiotics on kidney function broiler chicken.

Parameters	Treatments				
	T0	T1	T2	T3	T4
Creatinin (mg/dl)	0.16 ± 20.41 ^a	0.22 ± 18.18 ^{ab}	0.25 ± 15.32 ^{bc}	0.22 ± 31.49 ^{ab}	0.30 ± 13.33 ^c
BUN (mg/dl)	34.24 ± 3.79 ^a	38.00 ± 16.25 ^a	37.29 ± 15.61 ^a	42.71 ± 9.32 ^a	42.12 ± 13.34 ^a
Uric Acid (mg/dl)	4.33 ± 4.47 ^{bc}	4.61 ± 18.93 ^{cd}	3.73 ± 56.65 ^{ab}	3.14 ± 46.89 ^a	6.77 ± 44.23 ^d

Note: T-0 = control; T-1 = 100% fermented cow's milk; T-2 = 75% fermented cow's milk + 25% mung bean milk combination; T-3 = 75% fermented cow's milk + 25% soybean milk combination; and T-4 = 50% fermented cow's milk + 25% mung bean milk + 25% soybean milk. Duncan's test indicates significantly different (P<0.05) letters in the same column.

BODY WEIGHT

The average body weight of broiler chickens ranges from 958.75 to 1286.25 gram/chicken. The highest bodyweight was T2, 1286.25±20.93 gram/chicken and the lowest was T0, 958.75 ± 16.96 gram/chicken. The average highest to lowest consecutively, namely T2, T4, T3, T1, and T0. The body weight of broiler chickens increased from each treatment by 25.68% (T1), 29.33% (T3), 31.55% (T4), and 34.16% (T2) compared to T0.

The results were significantly different (P< 0.05) in enhancing broiler chicken body weight. T2 showed the highest body weight (P <0.05) compared to other treatments, except with T4. Body weight produced by T2 and T4 is in line with that achieved in body weight gain in the same treatment as a result of better levels of consumption and efficiency of ration digestion in livestock. According to [Adriani et al. \(2019\)](#), the increase in carcass weight is due to lactic acid bacteria colonizing on the surface of the digestive tract and producing metabolite products in the form of lactic acid and Short Chain Fatty Acid (SCFA), which can create an acidic atmosphere in the digestive tract with a pH of 4-5 ([Adriani et al., 2019](#)). T2 and T4 contain isoflavones in the form of aglycones derived from beans. This causes isoflavones and lactic acid bacteria to kill pathogenic microbes that stick to the digestive tract. This makes it easier for the body to absorb nutrients, which causes weight gain. Isoflavones from mung beans and soybeans increase body weight by acting as estrogens to improve growth performance and carcass weight without increasing abdominal fat ([Astuti et al., 2015](#); [Chen et al., 2016](#)). In addition to low body weight, the T0 exhibited high feed intake and weight gain ([Kumalasari et al., 2020b](#)). This demonstrates that providing basal ration without probiotics is inefficient for the feed consumed as meat, as nutrients are only partially absorbed.

CARCASS PERCENTAGE

The average carcass percentage ranges from 72.01 to 77.07%. The results of this study follow the opinion of North (1990) that the percentage of Ready Cook carcass is 70-77% of its live weight. The average of the highest to lowest carcass percentages, T2, T4, T3, T1, and T0, respectively. Based on the results of the Duncan test, dried probiotics

supplementation significantly (P<0.05) increased the carcass percentage. T2 gave the highest carcass percentage, but it was not significantly different from T4 (P> 0.05). The data showed that carcass percentage increases as body weight increases.

Lactobacillus bulgaricus, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* were used in the study. The growth rate of this lactic acid bacteria generates secondary metabolites, one of which is bacteriocin, an antimicrobial that effectively inhibits the development of pathogenic microbes requiring a pH of 6-7 ([Astuti et al., 2015](#)). Probiotics can both create and improve the activity of the protease enzyme. As a result, the broiler can make better use of the nutrients they absorb, allowing them to grow more quickly ([Balía et al., 2019](#)).

Probiotics in the diet increased the number of lysine analogs and aminoethyl cysteine produced by the digestive system. These molecules were transformed into the amino acids lysine and cysteine, improving protein retention, which is an essential step in meat production. Increasing the amount of protein and amino acids that are consumed through a ration can suffice the amount required by the metabolism, leading to an increase in tissue synthesis and a subsequent increase in both body weight and carcass weight ([Candrasih and Bidura, 2001](#); [Sari et al., 2019](#)). Positive nitrogen retention indicates weight gain due to muscle deposition. One of the factors affecting nitrogen retention is the health of the digestive tract.

Table 3 shows data on the supplementation of dry probiotics on the kidney function of experimental broiler chickens.

CREATININE

During a constant state of renal function, serum creatinine is commonly used to assess the glomerular filtration rate. Creatinine is derived from creatine, which is generated gradually in the kidneys and liver and then transported to the muscles, where it is stored as phosphocreatine. Creatinine is released into the bloodstream, transported to the kidneys for filtration, and ultimately eliminated via urine ([Park et al., 2013](#)).

Supplementation the average creatinine concentration of broiler chickens varies between 0.16 and 0.30 mg/dl. T0 showed the lowest creatinine level at 0.16 ± 20.41 mg/dl, while T4 showed the highest level at 0.30 ± 13.33 mg/dl. According to Hochleitner (2013), the typical range of creatinine levels is between 0.10 mg/dL to 0.40 mg/dL, and the average value of blood creatinine levels for all treatments is still within the normal range. This result showed that supplementation of dried probiotics to broiler chicken does not increase the average blood creatinine concentration above the standard limit. The analysis of variance showed that the supplementation of dried probiotics results in a significant difference ($P < 0.05$) in creatinine level. Duncan's test showed that T4 was significantly higher compared to other treatments. Changes in creatinine levels are an essential marker indicating the evaluation of chemical effects on the kidneys. High creatinine levels can be thought to be caused by impaired collective tubular epithelial cell transport function and decreased proximal tubular function (Valchev *et al.*, 2014). T4 consists of dried probiotic cow's milk, mung, and soybeans. This research aligns with Shimmi *et al.* (2014), which showed that giving peanuts to mice can increase blood urea and creatinine levels. This study supplementation probiotics with an additional formulation of mung beans and soybeans, which caused high creatinine level, thereby increasing protein metabolism and renal urea excretion rate (Tang *et al.*, 2014). Protein from nuts affects creatinine level, but not directly, because creatinine is synthesized from essential amino acids, such as arginine and glycine (Yosi and Sandi, 2014). In this study, the increase in creatinine and urea levels was still within normal limits, which showed a positive impact because it can indicate an increase in protein metabolism to support the process of protein formation in the growth process. In this study, the mungbeans and soybeans were fermented, which can produce beneficial isoflavones. Nagano *et al.* (2016) found that fermented mungbeans and soybeans provide more excellent health benefits than unfermented ones due to the increased bioavailability of isoflavones. Isoflavones are secondary metabolites produced by plants, which can function to prevent cell damage caused by radicals (Cahyati *et al.*, 2013). In conclusion, although T4 showed the highest creatinine level but still within normal limits. Apart from that, T4 also contains isoflavones resulting from fermented mungbeans and soybeans, which will maintain the integrity of kidney cells, thereby preventing damage.

According to Suardana (2007), LAB can produce an acidic environment because its metabolites are lactic. In addition to producing acetic acid, LAB can produce antimicrobials (bacteriocins, hydrogen peroxide, and diacetyl) that efficiently inhibit the growth of other undesirable bacteria that require a pH of 6-7. One approach to using probiotics

with Competitive Exclusion (CE Quality) involves the introduction of cultures of non-pathogenic bacteria into the animal's digestive system to minimize colonization or populations of dangerous bacteria. According to Langhout (2000), lactic acid can reduce bacterial toxins, change the shape of smooth cell walls, minimize the colonization of harmful bacteria, and improve animal health. This condition will increase the livestock's resistance to stress by increasing their body resistance.

BLOOD UREA NITROGEN

As a measure of kidney function, blood urea nitrogen (BUN) levels explain the equilibrium between urea production and protein catabolism. The mean blood urea of broiler chickens ranged from 34.24 to 42.71 mg/dl. Based on the analysis of variance, there was no significant difference ($P < 0.05$). One thing that influences the concentration of BUN is the consumption of protein in the diet. In this study, feed intake of protein for each treatment is the same, amounting to 21.40%, so the results are similar. Correlation between the high intake of feed containing many protein-forming precursors and the high level of protein catabolism that occurs in the liver, where supplementation of dry probiotics increases the production of protein-forming enzymes in the digestive tract and the absorption of these nutrients. This condition will increase the amount of protein and amino acids broken down in the liver through the urea cycle so that the resulting blood urea levels are high. According to Yosi and Sandi (2014), increased protein absorption is followed by increased BUN and creatinine levels due to protein metabolism. Even though it occurs indirectly, creatinine is produced by forming creatine from the necessary amino acids, arginine and glycine. An increase in BUN and creatinine levels within the normal range is the logical result of increased protein metabolism, which is required to support the synthesis of enzymes whose primary precursor is protein. The BUN concentration in this study shows that the kidney condition is normal.

URIC ACID

Uric acid is an insoluble nitrogen waste deposit that land snails, insects, birds, and some reptiles excrete. This happens because these species do not have the uricase enzyme. According to Sastiani (2004), uric acid is a normal metabolism of protein digestion or the breakdown of purine compounds that should be excreted through the kidneys, feces, or sweat. The average blood uric acid of broiler chickens ranged from 3.14 to 6.77 mg/dl. These results are the same as those reported by El-Katcha *et al.* (2014), a range of uric acid in chickens is around 4.87-6.57 mg/dl and is still included in the normal category. Based on the analysis of variance, there was no significant difference between treatments ($P > 0.05$). Based on the Duncan test

T2 and T3 were significantly lower ($P < 0.05$) than T4. The T4 treatment is a combination of fermented cow's milk, fermented mung bean, and fermented soy milk, where two nut species are potential sources of purines because it is logical that T4 shows the highest yield of uric acid. While T2 and T3 only uses one of nut. In mammals, urea levels in the blood signal protein metabolism, whereas in chickens, uric acid is the primary end product of protein metabolism (Golstein and Skadhauge, 2000). This is due to the inability of poultry to manufacture arginase, a key enzyme in the urea cycle. The kidneys excrete uric acid, which the liver produces as a byproduct of nitrogen or protein metabolism. Uric acid is not toxic or dangerous in the chickens' bodies, but if it forms uric crystals, it will damage the body's tissues. This happens because it is not immediately excreted out of the body due to kidney problems (Sulistyoningsih and Rakmawati, 2015).

CONCLUSIONS AND RECOMMENDATIONS

The effect of dried probiotics on growth performance, including feed conversion ratio, body weight, and carcass percentage, was not statistically significant but did increase relative to the control group. The T2 and T4 treatments increased body weight and carcass. The effect of dry probiotics on growth performance, including feed conversion ratio, body weight, and carcass percentage, was not significant statistically but did increase relative to the control group. The T2 and T4 treatments increased body weight and carcass.

Overall, the supplementation of probiotics does not have a bad effect on kidney health as seen in the concentration creatinine, BUN, and uric acid levels, which are still within normal levels. Need to be tested and re-examined regarding this result of controversy with the observations of other researchers.

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NOVELTY STATEMENT

The novelty of this research is that the use of probiotics in dry form containing a fairly complete consortium of microbes, namely *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*, is associated with kidney function in broilers as

seen from the parameters creatinine, BUN, and uric acid.

AUTHOR'S CONTRIBUTION

DL: statistical analysis and editing the manuscript

CK: writing and editing the manuscript, laboratory analysis, statistical analysis.

LA: idea and research design, statistical analysis

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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