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EFFECT OF USE OF PREBIOTICS^{BLS} (*Bacillus licheniformis, Lactobacillus sp.* and *Saccharomyces cerevisiae*) BASED ON SHRIMP WASTE ON PROTEIN EFFICIENCY RATIO IN INDONESIA LOCAL CHICKEN

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Abstract

One of the efforts to improve feed quality is by using BLS (Bacillus licheniformis., Lactobacillus spp. and Saccharomyces cerevisiae) microbial services from the shrimp waste substrate, hereinafter referred to as Prebiotics^{BLS}. BLS microbes are probiotic organisms that can improve the performance of livestock effectively (growth-promoter). Prebiotics^{BLS} is expected to function as an emulsifier in increasing nutrient absorption and metabolism so that it is effective in converting feed proteins into growth in Indonesia local chickens. The aim of the study was to determine the response of Prebiotics^{BLS} in feed to the performance of Indonesia local chickens. The study used 120 local one-day old chickens which were divided into 24 cage units, each cage unit consisted of 5 chickens, and kept for eight weeks. The experimental design used was a completely randomized design, consisting of 6 feed treatments (R0 = basal feed / CP 15%; R1 = basal feed + 1.0% Prebiotics^{BLS}; R2 = basal feed + 1.5% Prebiotics^{BLS}; R3 = basal feed + 2.0% Prebiotics^{BLS}; R4 = basal feed + 2.5% Prebiotics^{BLS}; and RS = standard feed / CP 18%), each treatment was repeated four times. The variables observed were feed consumption, protein consumption, body weight gain, and protein efficiency ratio. Data obtained from the results of the study were analyzed using analysis of variance, and differences between treatments were tested using Duncan's Multiple Distance Test. The experimental results showed that Prebiotics^{BLS} (based on shrimp waste) can be used as a feed supplement in the local chicken feed formula, and the use of Prebiotics^{BLS} at the level of 1.5-2.0% in feed (R4) is equivalent to the standard ration (RS / CP 18%).

***Key words**: Prebiotics^{BLS}, shrimp waste; microbes; protein efficiency ratio; local chicken.*

INTRODUCTION

Indonesia Local chicken is one type of local poultry that has the potential to produce meat and eggs which is widely cultivated by the community, especially those who live in rural areas. This is because local chickens adapt well to the environment. Consumer demand for local chicken meat is increasing every year. Based on data from the Direktorat Jenderal Peternakan (2014), the number of local chicken meat production from 2007 to 2014 has seen an increase, namely in 2007 as many as 294,889 tons to 332,095 tons in 2014. Noting the data, farmers must pay attention to the age of harvest from local chickens to meet market demand by paying attention to the efficiency of the ration used to produce high body weight gain.

Most local chickens consume rations to meet their protein and energy needs. The protein content in the diet is very influential in achieving body weight in Indonesia local chickens. The protein content in the ration is needed for tissue growth, tissue repair, and production management as well as part of the structure of the enzyme, so that protein is known as one of the main constituents of body cells and tissues (Degusa, 2002; Close and Menke, 1986). This shows that protein plays an important role in achieving the desired body weight.

Provision of rations with good quality protein can certainly affect the level of growth and development of local chickens. The body weight gain produced is a picture of the quality of rations given. Body weight gain results from the high quality of protein consumption. High protein quality will affect protein intake into meat so that amino acids are fulfilled in the body. Body weight gain is directly caused by the availability of tissue-forming amino acids, so the consumption of protein rations is directly related to the growth process.

Protein quality is determined by feed ingredients that make up rations, especially feed ingredients that are commonly used in Indonesia, namely fish meal. Fish meal has a high nutrient content, especially in the protein content (by 58%) which affects the quality of protein in chicken rations (Widodo, 2010; Wahju, 1997). However, the price of fish meal is expensive, and its availability is limited, it is necessary to look for alternative protein sources of feed ingredients that are cheap, easy to obtain, abundant availability, and have high protein content. The material is shrimp waste whose quality is expected to match the quality of ration from the use of fish meal.

Shrimp waste is a fishery waste whose numbers are increasing along with the increase in shrimp exports. Shrimp processing businesses in Indonesia have a production capacity of around 500,000 tons per year, out of the total production, 80 - 90% are exported in the form of head and skin frozen shrimp. Head and skin weight reach 60 - 70% of total weight (Direktorat Jenderal Budidaya Departemen Perikanan dan Kelautan, 2005). The volume of waste (head and shrimp skin) produced can reach 203,403 - 325,000 tons per year. This amount is a large enough potential to be used as a feed ingredient for protein in the preparation of local poultry rations.

The specialty of shrimp waste is that it has a good nutrient content, especially protein (42.65%), which almost matches fish meal (Gernat, 2001). Constraints in the utilization of shrimp waste are the limiting factor in chitin. Chitin binds to proteins and minerals in covalent glucosides bonds, making it difficult to digest by the poultry digestive enzymes (Abun et al., 2012; Abun, 2008). Therefore, an effort is needed to improve its quality so that it can be used as feed ingredients in the preparation of poultry rations.

One effort to improve the quality of feed ingredients is by microbiological processing through gradual fermentation techniques using *Bacillus licheniformis, Lactobacillus sp*., and *Saccharomyces cerevisiae*, and supplemented with Cu, Mo, and Se minerals during bio-process (Prebiotics^{BLS}). Bio-process products (Prebiotics^{BLS}), function as emulsifiers in the digestive tract of chickens, thereby increasing absorption and efficiency of nutrients (growth-promoters). *Bacillus licheniformis* bacteria produce chitinase and protease enzymes with deproteination properties which can free up some nitrogen or proteins from chitin bonds (Austin, 1988; Austin et al., 1981). *Lactobacillus sp*. serves to break down glucose, sucrose, maltose, and lactose, and the process of mineralization (Cira et al., 2000; Banwart, 1989). *Saccharomyces cerevisiae* is a yeast that produces the enzymes amylase, lipase, protease, and other enzymes that can help digestion of nutrients in the digestive organs (Bisping, 2005; Lee and Tan 2002). The technology of shrimp waste fermentation is one of the practical processing alternatives, and the results are favored by livestock, the price is low, and the value of nutrients is increased (especially protein), thus affecting the quality of protein rations.

One way to assess the quality of protein rations is to calculate the value of protein efficiency ratio. Protein Eefficiency Ratio (PER) is a method used to determine the quality of protein rations and is interpreted as increasing body weight divided by protein consumed (Leeson and Summers, 2001; Anggorodi, 1994). The protein efficiency ratiodetermines the level of efficiency of a livestock in changing each gram of protein into several body weight growths.

MATERIALS AND METHODS

Making Prebiotics^{BLS}

The materials used in this experiment include: Shrimp waste, Isolate *Bacillus. licheniformis, Lactobacillus sp*. and *Saccharomyces cerevisiae*, aquadest, glucose, yeast extract, tripton, NaCl, NaOH, $CaCO_3$, pH4 buffer, pH7 buffer, pH9 buffer, and Bovin Serum Albumin. The tools used are jars of stenles (reactors), water bath, shaker bath, autoclave, goblets, Bunsen burners, Petri dishes, porcelain dishes, centrifuges, funnels, PH-meters, spectrophotometers, test tubes, kilns, and containment machines. The stages of bio-process are as follows:

Deproteination. Perform fermentation at the Autho-Shaker-Bath (ASB). Shrimp waste is put into a jar of stainless, then inoculated with

Bacillus licheniformis inoculum at a dose of 2% (volume/ weight). Then put into ASB machine for 2 days at 45^0C with a rotation of 120 rpm (Abun et al., 2016).

Demineralization. Deproteination products, then added 2% (volume/weight) *Lactobacillus sp* inoculum, then incubated for 2 days at 35^0C, using ASB machines with 120 rpm rotation (Abun et al., 2016).

Fermentation with *Saccharomyces cerevisiae*. The demineralization products were then fermented using *Saccharomyces cerevisiae* 3% (volume/weight), then incubated for 2 days at 30^0C, using ASB machines with 120 rpm rotation (Abun et al., 2016).

Bindering. Bio-process products are then supplemented with cuprum (Cu) minerals, molybdenum (Mo) and selenium (Se) of 0.15 ppm, and additional energy sources (corn flour). Then the milling with a particle size of 60 mash. The product is Prebiotics^{BLS} (feed supplement) (Abun et al., 2018).

Feeding Trial

The study used 120 Sentul day-old (DOC) type local chickens without straight runes obtained from the Development Centre for Poultry Breeding, Jatiwangi, Majalengka-West Java, Indonesia. DOC has an average coefficient of variation of initial weight of 8%. The cage used is a cage-shaped cage of 24 units with a length of 0.7 m, a width of 0.5 m, and a height of 0.7 m. Each cage unit consists of 5 chicks and is equipped with a feed place in the form of a round feeder and a round water drinking water place made of plastic, and a 15-watt incandescent lamp. Chicken maintenance is carried out from 1 day to 8 weeks, giving rations and drinking water is done in *ad libitum*.

The feed ingredients for the ration consist of yellow corn, fine bran, soybean meal, fish meal (included in RO and RS), CaCO3, and Prebiotics^{BLS}. Basal ration (R0) and standard ration (RS) were prepared based on Indonesia's national standard recommendations (1995) and Zainuddin et al. (2004). The protein and energy content for basal ration (R0) is 15% and 2750 kcal/kg, and the standard ration (RS) is 18% and 2750 kcal/kg. The treatment ration is as follows:

R0 = basal / protein ration 15%;
R1 = basal ration + 1.0% Prebiotics ^{BLS};
R2 = basal ration + 1.5% Prebiotics ^{BLS};
R3 = basal ration + 2.0% Prebiotics ^{BLS};
R4 = basal ration + 2.5% Prebiotics ^{BLS}; and
RS = 18% standard / protein ration.

Prebiotics^{BLS}, treatment rations and local chicken can be seen in Figure 1.

Figure 1. Prebiotics ^{BLS}, Treatment Rations, and Indonesia Local Chickens (Sentul chickens)

The study was carried out using the experimental method and used a completely randomized design (CRD) with 6 types of ration treatment and repeated 4 times. The ration treatment consisted of R0 = basal ration / CP 15%; R1 = basal ration + 1.0% Prebiotics^{BLS}; R2 = basal ration + 1.5% Prebiotics^{BLS}; R3 = basal ration + 2.0% Prebiotics^{BLS}; R4 = basal ration + 2.5% Prebiotics^{BLS}; and RS = standard ration / CP 18%. The data obtained were analyzed Varian (F Test) and the differences between treatments were tested using Duncan's Multiple Distance Test.

RESULTS AND DISCUSSIONS

The average results of the study in the form of feed consumption, protein consumption, body weight gain, and protein efficiency ratio of each local chicken from each treatment during the experiment are presented in Table 1.

Table 1. Average Feed Consumption, Protein Consumption, Weight Gain, and Protein Efficiency Ratio in Indonesia Local Chickens

Variable	Treatment					
	RO	R1	R2	R3	R4	RS
Feed consumption (g/bird)	2892.69 A	2845.04 A	2872.59 A	2849.42 A	2817.76 A	2913.61 A
Protein consumption (g/bird)	433.90 B	426.76 B	430.89 B	427.41 B	422.67 B	524.45 A
Weight gain(g/bird)	1068.08 B	1067.33 B	1130.00 A	1109.39 AB	1073.55 B	1149.26 A
Protein Efficiency Ratio	2.46 B	2.50 B	2.62 A	2.60 A	2.54 AB	2.19 B

Description: R0 = basal ration / CP 15%; R1 = basal ration + 1.0% Prebiotics^{BLS}; R2 = basal ration + 1.5% Prebiotics^{BLS}; R3 = basal ration + 2.0% Prebiotics^{BLS}; R4 = basal ration + 2.5% Prebiotics^{BLS}; and RS = standard ration / CP 18%.

The use of Prebiotics^{BLS} in the ration did not affect the consumption of rations, but it affected the protein consumption, weight gain, and protein efficiency ratio (Table 1). Indonesia Local chicken protein consumption in treatment RS was significant ($P<0.05$) higher than other treatments, but between treatments R0, R1, R2, R3, and R4 did not show significant differences ($P>0.05$). Indonesia Local chicken weight gain in RS was not significantly different ($P>0.05$) with R2 and R3 treatment, but it was significant ($P<0.05$) higher than R0, R1, and R4 treatments. The protein efficiency ratio of Indonesia local chicken in the R2 and R3 treatments showed no significant difference ($P>0.05$), but it was significant ($P<0.05$) higher than the treatment of RS, R0, and R1.

The use of Prebiotics^{BLS} based on shrimp waste up to the level of 2.5% in the ration, did not affect the palatability of Indonesia local chickens, but it gave a positive effect on increasing body weight and protein efficiency ratio. This shows that Prebiotics^{BLS} has better nutritional value, especially organic acids and enzymes produced by microbes *BLS / Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae.* (Collins and Gibson, 1999). Organic acids and enzymes are needed by chickens for growth. The use of Prebiotics^{BLS} at the level of 1.5-2.0%, optimal in achieving weight gain and protein efficiency ratio in Indonesia local chickens.

Feed consumption is strongly influenced by the palatability of feed ingredients. As stated by McDonald et al. (1981) and Leeson and Summers (2001) that palatability is an important factor that determines the level of ration consumption, and palatability depends on the odor, taste, color, and texture of feed ingredients. The use of Prebiotics^{BLS} based on shrimp waste did not cause a decrease in feed consumption compared to basal ration or standard ration (R0 and RS). This indicates that the use of Prebiotics^{BLS} to the level of 2.5% in the ration does not cause odor, taste, color, and texture that is not liked by local chickens.

The physical structure of ration constituent feed ingredients also determines the amount of consumption of rations. The chemical structure of chitin is like cellulose, with the bonds that occur between the monomers strung together with glucoside at the position of β (1-4). The difference with cellulose is that the hydroxyl group bound to carbon atom number two is replaced by the acetamide group ($NHCOCH_3$) in chitin so chitin becomes an N-acetyl glucosamine-based polymer (Muzzarelli, 2000; Muzzarelli and Joles, 2000). Chitin monomer units have a molecular formula $(C_8H_{13}NO_5)_n$ with levels of C, H, N and O respectively 47.29%, 6.45%, 6.89% and 39.37%(Zakaria et al., 1995; Williams and Shih 1989; Tsugita, 1990). The chemical structure provides an illustration of the physical form of chitin from tiger shrimp waste. Maynard and Loosely (1978) asserted that the condition of the physical structure of feed rationing ingredients determines the amount of consumption of rations. The same thing was also expressed by Scott et al. (1982) that a large amount of ration consumption is determined by the physical properties of feed ingredients. Other factors that affect ration consumption are digestive tract capacity and digesta movement (Sibbald and Morse, 1983; Sklan and Hurwitz, 1980), gender, daily activities, quality and quantity of rations, and physical form of rations (NRC, 1994), size and nation of chickens, environmental temperature, production stage, and energy in the ration (Wahju, 1997).

A good amino acid balance in the ration is shown by obtaining optimal body weight gain, this illustrates an improvement in the quality of protein rations with the addition of

Prebiotics^{BLS}. Deproteination by *Bacillus licheniformis* which produces the enzyme chitinase and protease enzyme to degrade β (1,4) glyosidic bonds in chitin and free some proteins in the form of N-Acetyl-D-glucosamine and acetyl amino monomers thereby increasing protein digestibility (Rahayu et al., 2004; Kanauchi et al., 1995), *Lactobacilus sp*. bacteria which functions in the demineralization process, and breaks down glucose, sucrose, maltose, and lactose into lactic acid (Lee and Tan, 2002). Fermentation with the help of *Saccharomyces cerevisiae* yeast that produces amylase, lipase, protease, and other enzymes can help digestion of nutrients in the digestive organs (Wagstaff, 1989).

The consumption of protein in the R4 treatment was significantly higher compared to other treatments. This is caused by the protein content of the ration at treatment R4 (protein 18%) higher than the other treatments (protein 15%). High protein consumption does not always have a positive effect on growth and the protein efficiency ratio (Iskandar et al., 2001; Rosenfeld et al., 1997). Tissue protein synthesis is largely determined by the completeness and level of amino acids that come or are transported into the tissue cells. In accordance with the opinion of Maynard and Loosely (1978), that the synthesis process that takes place inside the ribosome is very dependent on the presence of amino acids needed and comes picked up by DNA into the tissue. This causes the ration with the addition of Prebiotics^{BLS} to produce better body weight compared to without Prebiotics^{BLS}, although the protein content of the diet is relatively lower.

Prebiotics^{BLS} based on shrimp waste with *Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae* microbes can improve the quality of protein rations by increasing the completeness and balance of essential amino acids contained in it. The resulting protein efficiency ratio is higher than the results of Wiradisastra (1986) study, which states that the protein efficiency ratio for 8 weeks old chickens is 1.72-1.93. This is caused by differences in the types of chicken used and differences in protein content of rations. In accordance with Wahju (1997) opinion, the protein efficiency ratio is influenced by age, type of chicken, sex, duration of experimentation, and protein ration level. The difference in protein efficiency ratio values is caused by the presence of shrimp-based Prebiotics^{BLS}.

Bio-process shrimp waste by *Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae* can improve the quality of protein ration by increasing the completeness and balance of essential amino acids contained (Rao et al., 1998; Reddy and Quddratullah, 1996), so Prebiotics^{BLS} can be used as a feed supplement in Indonesia local poultry feed formulas. The balance of amino acids, especially methionine and lysine in the treatment ration with the addition of Prebiotics^{BAS} at the level of 1.5-2.0%, is in the ideal balance (methionine:lysine = 0.49-0.52:1) In line with Widodo (2010), that the balance of the amino acids methionine and lysine in the ration formula is between 0.48-0.52:1. This illustrates that the optimal level of use of Prebiotics^{BLS} is 1.5-2.0% in the Indonesia local chicken feed formula, which results in the highest growth and protein efficiency ratio.

CONCLUSIONS

Based on the results of the research and discussion it can be concluded that the use of shrimp waste-based Prebiotics^{BLS} at the level of 1.5% is optimal in increasing growth and protein efficiency ratio in Indonesian local chicken.

Prebiotics^{BLS} based on shrimp waste can be used as feed supplement in Indonesian local chicken feed formulas, and its use is between 1.5-2.0%.

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