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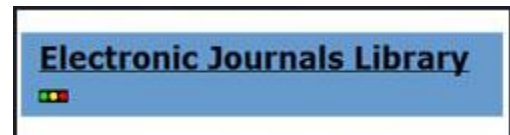
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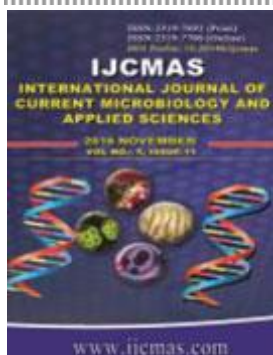
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Implementation Probiotics Cellulolytic B-7 Bacteria (Isolation from Buffalo Rumen) into Rations on the Performance, Abdominal Fat and Serum Cholesterol of Duck

**Eny Puspani\***, **Desak Putu Mas Ari Candrawati** and **Dan I.G.N.G. Bidura**

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\**Corresponding author*

## Abstract:

This research aims to study the influence of cellulolytic B-7 bacterial isolates rumen of buffalo as a source of probiotics on the performances, abdominal fat, and cholesterol serum of a male Bali duck. A total of 240 male bali ducks were randomized in a completely randomized design (CRD) with four treatments and six replications. Treatment is attempted is the ducks that were given rations without supplementation of probiotic cultures cellulolytic as control (A); ration with 0.20% (B); 0.40%; and 0.60% probiotic cultures selulolyticy B-7, resvectively. The isolate has passed the test and has been considered as potential probiotic according to our previous study (Bidura *et al.*, 2014). The results showed that the ducks were given rations containing probiotics result in weight gain and feed efficiency significantly ( $P < 0.05$ ) better than the control. However, administration of probiotics through diet significantly ( $P < 0.05$ ) can reduce abdominal fat weight and serum kolseterol in ducks. From these results it can be concluded that supplementation of 0.20% to 0.60% probiotic cellulolytic B-7 bacteria were isolated from the rumen of buffalo in the ration can significantly improve live weight gains and feed efficiency of Bali duck. In contrast, significantly reduced abdominal-fat weight and serum cholesterol of ducks.

**Keywords:** Probiotics, buffalo rumen, performance, cholesterol, abdominal-fat, duck.

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Original Research Article

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## Implementation Probiotics Cellulolytic B-7 Bacteria (Isolation from Buffalo Rumen) into Rations on the Performance, Abdominal Fat and Serum Cholesterol of Duck

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### ABSTRACT

This research aims to study the influence of cellulolytic B-7 bacterial isolates rumen of buffalo as a source of probiotics on the performances, abdominal fat, and cholesterol serum of a male Bali duck. A total of 240 male Bali ducks were randomized in a completely randomized design (CRD) with four treatments and six replications. Treatment is attempted is the ducks that were given rations without supplementation of probiotic cultures cellulolytic as control (A); ration with 0.20% (B); 0.40%; and 0.60% probiotic cultures cellulolytic B-7, respectively. The isolate has passed the test and has been considered as potential probiotic according to our previous study (Bidura *et al.*, 2014). The results showed that the ducks were given rations containing probiotics result in weight gain and feed efficiency significantly ( $P < 0.05$ ) better than the control. However, administration of probiotics through diet significantly ( $P < 0.05$ ) can reduce abdominal fat weight and serum cholesterol in ducks. From these results it can be concluded that supplementation of 0.20% to 0.60% probiotic cellulolytic B-7 bacteria were isolated from the rumen of buffalo in the ration can significantly improve live weight gains and feed efficiency of Bali duck. In contrast, significantly reduced abdominal-fat weight and serum cholesterol of ducks.

### Keywords

Probiotics,  
buffalo rumen,  
performance,  
cholesterol,  
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### Introduction

Public attention to the fat and cholesterol become greater, especially after it emerged that the consumption of fat/cholesterol excess will affect health. Not only to the increase in coronary heart disease, but lately informed also against cancer, diabetes, and high blood pressure (Santoso and Sartini, 2001). It was also reported that accumulation of high fat in the stomach and viscera may reduce the benefits of post-

harvest plant and improve wastewater treatment problem. Therefore, it is very helpful if it can reduce cholesterol and abdominal fat chicken with biotechnology utilizing probiotics.

The use of antibiotics to stimulate the growth of poultry has been banned in European and American countries (Ahmad, 2006). Therefore, nutritionists and livestock

production attracted to compounds that can serve as a substitute for antibiotics. Probiotics is one approach that has the potential to replace antibiotics. Probiotics are live microorganisms which when administered through the digestive tract, have a positive impact on the health and production of the host. According to Putra *et al.* (2015), the addition of probiotics in the ration is expected to enhance the role of normal flora in the digestive tract of cattle to produce exogenous enzymes, such as amylase, protease, and lipase which can enhance the activity of endogenous enzymes to hydrolyze feed nutrients.

Probiotics contain beneficial bacteria species that is normally found in the intestinal tract. Studies show that consumption of certain probiotic strains, particularly *Lactobacillus* can alter the intestinal microflora to produce a beneficial effect. Most commercially available strains of *Lactobacilli* and *Bifidobacteria* species are generally considered safe and can be very helpful in the treatment of pediatric diarrhea. However, the clinical benefits of probiotic therapy depends on many factors, such as the type of bacteria, the accuracy of the dose, method of delivery, and other environmental factors underlying (Dinkçi *et al.*, 2006).

Based on this, interesting to note is the use of cellulolytic microbes from buffalo rumen fluid as the fiber degrading in the gastro intestinal tract of poultry. This is possible because the buffalo rumen fluid microbes turns out to have the highest cellulolytic activity compared with other livestock cellulolytic microbes. According to Sudirman (2004) in addition to determining the source of the microbial digestion of fiber activity, is also determined by the exact microbial inoculum dose, type, and microbial populations were used. Provision of microbial cultures from buffalo rumen

fluid to the ducks is expected to lead to a synergistic effect between species of buffalo rumen microbes with microbes of duck in digestive tract, which can lead to the ability to digest fiber.

Some of the results of the preliminary study on the use of probiotics in the ration was able to improve performance, digestibility, efficiency ration, and lowering the amount of abdominal fat and cholesterol body poultry (Bidura *et al.*, 2012; Bidura *et al.*, 2014; Bidura *et al.*, 2016; Candrawati *et al.*, 2014). According to Siti *et al.* (2016), supplementation of 0.40 to 0.60% cellulolytic bacteria culture isolated from the rumen of buffalo in the tofu-based rations can improve the performance, carcass weight, carcass percentage, and the percentage of breast meat of ducks. In contrast, significantly reduced the amount of abdominal fat, blood serum cholesterol levels, and the concentration of N-NH<sub>3</sub> in excreta of duck. Furthermore, the beneficial effect of supplementation of probiotics has also been reported by some research workers such as Yousefi and Karkoodi (2007), Yamada and Sgarbieri (2005), Queiroz *et al.* (2004) and Roni *et al.* (2014), Ghasemi *et al.* (2006), Mohiti *et al.* (2007). On the other hand, some authors such as Ayanwale *et al.* (2006) reported that probiotics supplementation on diet has no effect on performances.

Based on the description above research aimed to study the effect of supplementation cellulolytic bacteria selected from the rumen of buffalo into diets to improve performance and reduce levels of cholesterol of ducks.

## **Materials and Methods**

### **Animals and experimental design**

A total number of 240 male Bali duckling (*Anas sp*) at 2 weeks of age and with



homogenous body weight were randomly divided and caged in 24 separate pens with 10 birds in each pen. Prior to introduction of the experimental birds, the pens were thoroughly cleaned and disinfected. During the course of experiment, the birds were maintained under standard management condition for 42 days (6 weeks). For the purpose of examination of their carcasses and cholesterol concentration, 30 birds from each treatment group were randomly sampled.

A completely randomized design (CRD) was employed in the current experiment. Four treatment groups were assigned depending on their diets. All animals were fed an isonitrogenic and isocaloric ration in the form of mash and has been prepared in a such that meet the birds requirement to NRC (1984). Various levels of cellulolytic B-7 bacteria isolates obtained from rumen of buffalo as a source of probiotics slaughtered at the local abattoir, were supplemented on the diet. The difference in its level of application determined the four different experimental groups assigned. In the current experiment, addition of 0.0% (0 g/kg diet); 0.20% (2.0 g/kg diet); 0.40% (4.0 g/kg diet); and 0.60% (6.0 g/kg diet) were considered as the Treatment A (control), Treatment B, Treatment C, and Treatment D, respectively. Each group consisted of 6 replications with 10 birds in each replicate; therefore, the total number of experimental animals used in the current study was 240 birds. They were all provided with feed and drinking water *ad libitum*.

The probiotic used in the current study was cellulolytic B-7 bacteria isolates, which was isolated from from rumen of buffalo slaughtered at the local abattoir. The isolate has passed the test and has been considered as potential probiotic according to our previous study (Bidura *et al.*, 2014).

## **Body Composition**

At the end of the experiment (56 days of age) 30 birds from each treatment were selected and slaughtered for determination of body composition. The leg and breast meats were separated from the carcass. The parts of the body fat are: pad-fat (separated from the organs of the abdominal viscera to the skin), mecenteric-fat (linkage separated from the intestine), verniculus-fat, and abdominal-fat (a combination of fat-pad, ventriculus-fat, and mecenteric-fat).

## **Total serum cholesterol**

For analysis of total serum cholesterol, two ml of blood was taken from the jugular vein of each duckling and centrifuged at 3000 rpm for 20 minutes. The blood samples were allowed to clot in a sample bottle and serum harvested was used to determine the total serum cholesterol content with the aid of a analyzed by the method of Liberman-Burchard (1980).

## **Statistical analysis**

Data collected was subjected to Analysis of Variance and if significant different among the treatment group was noted, they were then underwent further statistical analysis following Duncan's Multiple Range Test (Steel and Torrie, 1989).

## **Results and Discussion**

Results of the current study are presented in Table 1. It can be noted that supplementation on diet of various level of the probiotic ranging from 0.20% to 0.60% resulted in a significant ( $P < 0.05$ ) increase in live weight gains. Moreover, the supplementation of 0.20-0.60% of *cellulolytic B-7* bacteria culture significantly ( $P < 0.05$ ) increased feed efficiencies.

However, no significant different ( $P>0.05$ ) in feed consumption was noted.

At the end of the experiment (at eight weeks of age), showed live weight gains during the six weeks of observation ducks that were given rations without supplementation of probiotic cellulolytic B-7 bacteris isolated from rumen buffalo as a control is 1058.75 g/birds (Table 1). Supplementation cellulolytic B-7 bacteria at the levels: 0:20%; 0:40%, and 0.60% in the ration, respectively were 18.48%; 19.56%; and 22.91% higher than controls and were statistically significantly different ( $P<0.05$ ).

The mean value of the feed conversion ratio (feed consumption: weight gain) on ducks control is 5.45 (Table 1). The mean value of FCR ducks received treatment B, C, and D, respectively, were: 14.13%; 14.50%; and 16.88% ( $P<0.05$ ) lower than the control. The results showed that the average amount of abdominal fat in ducks control was 2.85% body weight. The average amount of abdominal fat ducks received treatment B, C, and D respectively, are: 17.54%; 16.14%; and 18.60% ( $P<0.05$ ) lower than the control. Total cholesterol levels in the blood serum of control duck was 185.64 mg dl (Table 1). Mean serum cholesterol levels of ducks treatment B, C, and D, respectively, were: 11.22%; 10.61%; and 12.70% ( $P<0.05$ ) lower than the control.

The results showed that supplementation of 0.40 to 0.60% bacterial culture isolates cellulolytic B-7 were isolated from the rumen of buffalo in the diet can increase the performance of ducks (live weight gains and feed efficiency). Because culture isolate cellulolytic rumen of buffalo that have passed the test as an agent of probiotics and have activity cellulolytic or has an activity of CMC-ase (Bidura *et al.*, 2014) in the digestive tract is able to improve the

digestibility of the feed, in particular feed fiber roughness height, into compounds simpler so more easily absorbed by the body ducks.

Feed efficiencies and LWGs in bird offered probiotic group are higher than control groups. This may be caused by the fact that probiotics contain species of beneficial bacteria that are commonly found in the intestinal tract. Most commercially available strains of *Lactobacilli* and *Bifidobacteria* species are generally considered safe and may be especially helpful in treatment of diarrheal illnesses. However, clinical benefit of probiotic therapy is dependent on numerous factors such as type of bacteria, dosing regimen, delivery method, and other underlying host factors (Dinkçi *et al.*, 2006). The use of cultures such as *Saccharomyces cerevisiae* can improve weight gain, as a result of the response to increased dry matter intake. Especially, *Saccharomyces cerevisiae*, have been used in animal diets for several decades and are considered sources high quality proteins and B-complex vitamins, selenium and zinc (Queiroz *et al.*, 2004).

Moreover, Piao *et al.* (1999) noted that the use of 0.10% yeast (*Saccharomyces cerevisiae*) in the ration significantly increased weight gain, feed efficiency and nutrient digestibility as well as reduce the amount of N and P secreted in the feces. Study in ducks by Bidura *et al.* (2012) also confirmed the previous findings; supplementation of 0.10% yeast culture led to an improvement in feed intake, feed conversion ratio, and weight gain. Such a finding is further supported by results of Umiarti *et al.* (2014) who found that the use of 0.10% to 0.30% *Saccharomyces spp* culture significantly improved live weight gains and feed efficiency. More positive effects of yeast supplementation were

reported by Mulyono *et al.* (2009), Wu *et al.* (2005) and Huang *et al.* (2004). Santin *et al.* (2001) found that supplementation of feed with *S.cerevisiae* cell wall (0.2%) improved broiler body weight. Many studies indicate that the addition of the culture of probiotic or enzyme in high feed content NSP can significantly reduce the viscosity of the digestive tract (intestinal viscosity), increase energy and protein retention (Wang *et al.*, 2004; Bidura *et al.*, 2012; Chen *et al.*, 2005).

According to Stanley *et al.* (1993), which found that broiler chickens were given probiotics 0.10% can significantly improve weight gain and feed efficiency. Giving feed containing probiotics can stimulate the metabolism of poultry feed in the digestive process (Nurhayati, 2008). Probiotic microbes such as *Aspergillus oryzae* and *Saccharomyces cerevisiae* in the digestive tract of poultry at the level of 0.15% and 0.30% can increase the activity of amylolytic and proteolytic enzymes, thus increasing energy and digestibility of dietary protein termetabolis (Han *et al.*, 1999).

Apparently, the role of culture isolate cellulolytic rumen of buffalo as a probiotic agent in the digestive tract of ducks effective at levels from 0.20 to 0.60% in the ration. Piao *et al.* (1999) reported that probiotic supplementation in the diet can markedly increase live weight gains, nutrient absorption, and the absorption of nitrogen and phosphorus. The results of this study are supported by Wu *et al.* (2005) and Huang *et al.* (2004) that *Aspergillus xylanase* supplementation in wheat bran-based ration can improve the performance of broiler chickens. The same thing was reported by Mulyono *et al.* (2009) that the addition of 1.0% *S.cerevisiae* ( $9 \times 10^9$  cfu) derived from baker's yeast in the basal ration of broiler chickens significantly increase the digestibility of dry matter, digestible protein,

and protein efficiency ratio. Yi *et al.* (1996) reported that microbes in feed supplementation can memperbaiki N retention in broiler and improve the digestibility of protein. It was also reported by Chen *et al.* (2005), that the addition of probiotic complex (*L.acidophilus* and *S.cerivisae*) in the basal diet can markedly increase the digestibility of dry matter feed.

Utama (2011), reported that the administration of probiotics (yeast *Saccharomyces cerevisiae*) in feed can improve the digestibility of protein and crude fiber components, such as cellulose and hemicellulose, since been overhauled in the form of simple monosaccharide. Chen *et al.* (2005) reported that the complex probiotic supplementation in the ration can markedly increase the digestibility of nutrients. Many studies indicate that the addition of culture probiotic or enzyme in high feed content NSP's can significantly reduce the viscosity of the digestive tract (intestinal viscosity), increase energy and protein retention (Wang *et al.*, 2004; Bidura *et al.*, 2012; Yi *et al.*, 1996; Chen *et al.*, 2005).

Supplementation of 0.20 to 0.60% bacterial culture isolates cellulolytic B-7 were isolated from the rumen of buffalo in the diet significantly reduced the amount of abdominal fat and blood cholesterol levels of ducks. These results are supported by Nurhayati (2008) that the use of the feed mixture fermented by *Aspergillus niger* at the level of 10-30% significantly reduced abdominal fat weight. Lactic acid bacteria in the digestive tract of poultry is able to utilize the energy derived from carbohydrates to lower the pH of the digestive tract to 4.5 resulting in the digestive tract becomes acidic. Acidic conditions causing lipase activity to be limited, so that the reduced fat digestion and

subsequent synthesis of fat to decrease. Research on pigs conducted by Min (2006) reported that feeding fermented can reduce the fat content.

Supplementation of culture isolates B-7 cellulolytic bacteria isolated from the rumen of buffalo in the ration significantly reduced plasma cholesterol levels. According Ezema and Yeh (2015), probiotics may contribute to the regulation of serum cholesterol concentrations were carried out by deconjugated bile acids. Cholesterol is a precursor to the formation of bile acids and

bile acid excretion when deconjugated enhanced by probiotic supplementation, the precursor molecule then be required to restore the formation of bile acids. The results of this study are supported by Park *et al.* (2008) and Sutarpa *et al.* (2011) reported that the use of probiotics in the diet can lower cholesterol levels in serum and chicken meat. Probiotic supplementation at a rate of 1.0 g/kg ration can be recommended for optimum egg production and to reduce the concentration of cholesterol in serum and egg (Ezema and Eze, 2015).

**Table.1** Implementation of Probiotics cellulolytic B-7 bacteria isolates of Rumen Buffalo in rations for Reducing abdominal-fat and Cholesterol serum of Ducks (aged 2-8 weeks)

Variable	Supplemented of cellulolytic B-7 bacteria in rations (%)				SEM <sup>1)</sup>
	0.00	0.20	0.40	0.60	
Live weight gains (g/6 weeks)	1058.75b	1254.42a	1265.92a	1301.32a	52.056
Feed consumption (g/6 weeks)	5765.35a	5869.83a	5904.61a	5895.74a	78.457
Feed conversion ratio (feed/gains)	5.45a	4.68b	4.66b	4.53b	0.214
Abdominal fat (% berat badan)	2.85a	2,35b	2.39b	2.32b	0.107
Cholesterol serum (mg/dl)	185.64a	164.82b	165.95b	162.06b	5.038

Note:

1. Standart Error of the treatments means
2. Means with different superscripts within raws are significantly different (P<0.05)

Probiotic may contribute in the regulation of serum cholesterol concentrations conducted by deconjugated bile acids. As cholesterol is a precursor for bile acid formation and when deconjugated bile acids excretion is enhanced by probiotics supplementation, then more precursor molecules are needed for the recovery of bile acid formation (Ezema and Eze, 2015). Consequently, it may be expected that level of serum cholesterol decreases (Park *et al.*, 2008; Sutarpa *et al.*, 2011). Moreover, Klaver and Van Der Meer (1993) also suggested that co-precipitation with bile acids may be of

importance in decreasing serum cholesterol concentrations. Ezema and Eze (2015) suggested, probiotic (*S.cerevisiae*) inclusion level of 1.0 g/kg of layers mash is rekomanded for optimum hen-day egg performance and minimum serum and egg cholesterol content (Bidura *et al.*, 2016).

DeSmet *et al.* (1998) reported that probiotics could contribute to the regulation of serum cholesterol concentration by deconjugated bile acids. Since the excretion of deconjugated bile acid is enhanced and cholesterol is it's precursor, more molecules

are spent for recovery of bile acids (Ezema and Eze, 2015). As a result of increased synthesis of these acids, it is expected that the level of serum cholesterol will be reduced. That co-precipitation with bile acid might be of importance in decreasing serum cholesterol concentration.

In conclusion, that supplementation of 0.20 to 0.60% cellulolytic B-7 bacteria culture isolated from the rumen of buffalo in rations can improve the performance of ducks. In contrast, significantly reduced the amount of abdominal fat and blood serum cholesterol levels of duck.

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