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By

Siti, N.W., I G.N.G. Bidura, and I.A.P. Utami

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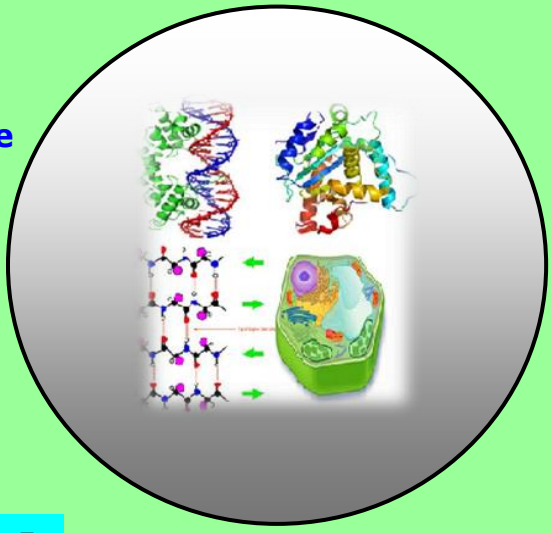

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Dr. N.W. Siti
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RESEARCH PAPER

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Siti, N.W., Igng Bidura, and I.A.P. Utami

Faculty of Animal Husbandry, Udayana University, Denpasar-Bali, Indonesia,
Jl. PB. Soedirman, Denpasar-Bali

ABSTRACT

This experiment was carried out to study the effect of supplementation culture cellulolytic bacteria (isolated from the rumen of buffalo) in the tofu-based ration on the growth performance of male Bali duckling up to ten weeks of age and N-NH_3 concentrations in excreta. One hundred and twenty of male Bali drakes (Anas sp) were assigned to 4 treatments in a completely randomized design. The treatments were the tofu-based rations without supplementation culture cellulolytic bacteria as a control (A); supplementing rations at 0.20% cellulolytic bacteria culture (B); by supplementing 0.40% cellulolytic bacteria culture (C); and 0.60% ration supplemented culture cellulolytic bacteria culture (D), respectively. The study showed that supplementation of 0.40 to 0.60% cellulolytic bacteria culture in tofu-based rations could improve significant differences (P<0.05) on live weight gain (LWG)s, carcass percentage, and were efficient in using feed compared than control (A). On the other hand, abdominal fat, serum cholesterol contents, and the concentration of N-NH_3 of the birds decreased significantly different (P<0, 05) than control. It was concluded that supplementation of 0.40 to 0.60% cellulolytic bacteria culture isolated from the rumen of buffalo in the tofu-based diets can improve on growth performance of male Bali duckling up to ten weeks of age. In contrast, significantly reduced the amount of abdominal fat, serum cholesterol contents, and the concentration of N-NH_3 in excreta.

Key words: Cellulolytic Bacteria, Probiotics, Tofu, Performance and Ammonia.

INTRODUCTION

Development of poultry in Indonesia is facing problems, the potential of the feed is not in accordance with the quantity, quality and continuity, so that needs serious attention, because feed is one of the success factors in the poultry business. Therefore, improved feed management is expected to improve business efficiency of poultry, especially duck. Enterprises poultry feed requires quite a lot, so it needs to be modified feeding using local feed ingredients with optimal benefits (Bidura, 2007).

Tofu is made from an extract of soy beans in the form of protein-rich wet waste (Wina *et al*., 2012). The main drawback tofu as poultry feed is the high water content (82-90%) and crude fiber (Wina *et al*., 2008). Tofu contains protein with amino acids lysine, methionine, and calcium is high enough. However, rough fiber content is high, so that it becomes a limiting factor use in poultry rations (Mahfudz, 2006).

Tofu waste, unlike soybean meal, is a cheap good protein source and readily available in Asia. Tofu is made from soybean by extraction and precipitation to produce a semi-solid product rich in protein (Wina *et al*., 2012). During the tofu preparation, the extracted protein, may or may not be heated, and is separated by filtration.

Interesting to be studied is the use of microbes in the rumen of buffalo, because most contain cellulolytic microbes and has a cellulolytic activity of the highest compared to other livestock cellulolytic microbes, such as cows (Prabowo *et al*., 2007). At the buffalo rumen fluid found seven cellulolytic bacteria colonies, while the four colonies in Bali cattle. Through the isolation and testing capabilities of microbial isolates selected as a probiotic and CMC-ase activity (Bidura *et al.,* 2014) and when implemented through a fermentation product continuously through feed, may be able to assist in the digestibility of poultry in the ration of fiber-based, from the aspect of value digestibility of dry matter, organic matter, and crude fiber.

Availability of feed tofu is a lot, especially in the industrial centers of domestic manufacture of tofu. Sometimes tofu is often posed a dilemma smell less pleasant, so disturb the environment. This is due to extremely high water content of tofu, so it is easy to rot (Bidura, 2007). It would be very wise if the tofu is used as feed ducks. Before given, first fermented tofu with inoculant that is able to act as probiotic microbes in the digestive tract of ducks (pass the test pH, temperature, acid and bile salts). Therefore, to take advantage of tofu, it must first be treated and one of them is by using a fermentation technique probiotic microbes.

Cellulose fraction is the most substantial component of a cell wall constituent of tofu, which is about 40-50%, which is very difficult/can not be digested by the digestive enzymes in duck. In order to be used, the cellulose must first be broken down into low molecular weight compounds, such as mono, di and tri-saccharides. The degradation involves complex cellulase enzymes produced by microbes (Wainwright, 2002), including the enzyme endo-beta-glucanase and beta-glucosidase.

Interesting to study is the use of cellulolytic microbes selected from buffalo rumen fluid as the fiber degrading microbes in the tofu before it is given to duck. This is possible because the bacteria from rumen fluid buffalo has a cellulolytic activity of the highest compared with other livestock cellulolytic microbes, such as Bali cattle (Prabowo *et al.,* 2007).

According to Sudirman (2011), in addition to a source of microbes that determines the activity of fiber digestion, is also determined by the precise dose of microbes, uniformity type, and microbial populations were used. Giving cellulolytic bacteria isolated from rumen fluid buffalo for ducks is expected to lead to a synergistic effect between cellulolytic bacteria species of buffalo rumen microbes in the digestive tract of ducks, so the ducks can improve the ability to digest fiber feed.

Fermented feed product can significantly increase growth and decrease serum cholesterol and improve carcass quality duck (Bidura *et al*., 2008). Other properties of fermented products is able to suppress the activity of the enzyme 3-hydroxy-3-methylglutaryl Co-A reductase which serves for the synthesis of cholesterol in the liver (Tanaka *et al*., 1992), and can reduce the amount of body fat of broilers (Kataren *et al.* , 1999). According Harmayani (2004), a bacterium that can grow and assimilate cholesterol in the small intestine has potential as a controller of the host blood serum cholesterol levels, as occurs in the small intestine cholesterol absorption process. The ability of assimilation of cholesterol by the probiotic bacteria vary between strains and requires anaerobic conditions and the presence of bile acids.

Based on the description above research aimed to study the effect of supplementation cellulolytic bacteria selected from the rumen of buffalo into tofu-based diets to improve performance and reduce levels of ammonia in the excreta of ducks.

MATERIAL AND METHODS

Design of Experiments

One hundred and twenty of male Bali drakes (Anas sp) were assigned to 4 treatments in a completely randomized design. Each treatment consisting of six replications with five birds per replication. The treatments were the tofu-based rations without supplementation culture cellulolytic bacteria as a control (A); supplementing rations at 0.20% cellulolytic bacteria culture (B); by supplementing 0.40% cellulolytic bacteria culture (C); and 0.60% ration supplemented culture cellulolytic bacteria culture (D), respectively. The average body weights of the four treatment groups were not significantly different at the beginning of the experiment. All birds were fed experimental diets for five weeks. The four experimental diets were based on tofu.

Rations and Drinking Water

Rations were used in this study was calculated based on the nutrient composition tables according to NRC (l984), using the feed, such as yellow maize, fish meal, coconut meal, rice bran, salt, and tofu. All diets in mash form and prepared by iso-energy (2900 kcal ME/kg) and iso-protein (17% CP). Diet and water were given *ad libitum* during the five-week trial period. Body weight and feed intake recorded weekly.

Tofu

Tofu is obtained from domestic industrial manufacturing know in the area Ubung Kaja, Denpasar. Feeding is done ad libitum. Given drinking water is well water obtained in the study by providing *ad libitum*

Materials and Research Proposition

The material used is cellulolytic bacteria selected from the rumen of buffalo. Selection and test the ability of probiotic agents and CMC-ase activity carried out at the Laboratory of Biosciences, University of Udayana, Denpasar-Bali (Bidura *et al*., 2014).

Chemicals used in this study are: bacterio logical solution peptone 0.1%, distilled water, nutrient broth, NaOH, H2SO4, glucose, glycerol, NaDC, and alcohol.

Culturing cellulolytic bacteria isolated from Rumen Buffalo

Culturing cellulolytic bacteria were isolated from the rumen of buffalo is done by isolation of cellulolytic bacteria rumen fluid buffalo. Isolates were obtained subsequently made cellulolytic bacterial culture using solid media (rice bran), namely: 150 g of molasses, 15 g of urea, 5 g of chalk, 5 g of salt, 2 g multi vitamin-mineral, 400 g rice bran, and water taste until the mixture reaches a weight of 1 kg, then add isolate cellulolytic bacteria that have been isolated from buffalo rumen fluid as much as 0.50%. Subsequently the mixture was incubated in an incubator space in anaerobic atmosphere for 1 week at a temperature of 37-39^{0}C (the temperature is kept constant). After one week of incubation, and then dried in an oven at a temperature of 45^{0}C and after dried crushed back and observed the number of cellulolytic bacteria colony on the culture (ready for use as a bacterial culture cellulolytic rumen fluid from a buffalo).

Table 1. Formula and Chemical Composition of Treatment Diets of Growing Male Bali Duckling up to ten Weeks old.

Diets		Supplemented cellulolytic bacteria culture (%) in diets			
		0.00	0.20	0.40	0.60
Ingredients (%):					
Yellow corn		47.20	47.20	47.20	47.20
Rice bran		10.50	10.50	10.50	10.50
Coconut meal		4.30	4.30	4.30	4.30
Fish meal		7.50	7.50	7.50	7.50
Tofu		***30.00***	***30.00***	***30.00***	***30.00***
Salt (NaCl)		0.50	0.50	0.50	0.50
Total		*100*	*100*	*100*	*100*
*Chemical composition **):*					
Metabolizable energy	(kkal/kg)	2900	2900	2900	2900
Crude protein	(%)	17.0	17.0	17.0	17.0
Crude fibre	(%)	9.01	9.01	9.01	9.01
Eter extract	(%)	4.78	4.78	4.78	4.78
Calcium	(%)	0.94	0.94	0.94	0.94
P-available	(%)	0.61	0.61	0.61	0.61
Argynine	(%)	1.64	1.64	1.64	1.64
Lysine	(%)	1.41	1.41	1.41	1.41
Metionine+systeine	(%)	0.81	0.81	0.81	0.81

Notes: 1. Based on the calculation according to Scott *et al*. (1982)

Body Composition

At the end of the experiment (70 days of age) 12 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), vernticulus-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 cholesterol content of blood serum was analysed according to Plummer (1977).

Concentration of N-NH3

The method used is the method of Phenolhypoclorite in Saransi *et al.* (2004). Gastrointestinal fluid sampling is done at the end of the study. Gastrointestinal fluid that is in the digestive tract in the cecum and colon removed, then filtered with satin triplicate into a test tube which had previously been spilled one drop of concentrated sulfuric acid. Samples were then taken to the laboratory for analysis of N-Ammonia content.

Statistical Analysis

All data were subjected to a one-way analysis of variance test (Steel and Torrie, 1989). Statistical significances among treatment means were determined by method of New Multiple Range Test of Duncan when the F value was significant at 5% level.

RESULT

At the end of the experiment (at eight weeks of age), mean final body weight by the end of the duck tofu-based diet with the addition of 0.20% cellulolytic bacterial culture in the ration (B), did not show any significant differences (P>0.05) with the controls. However, the addition of 0.40% buffalo rumen cellulolytic bacterial culture in the ration (C) and 0.60% in the diet (D), respectively 13.14% and 11.22% (P<0.05) more higher than the control.

The average value of FCR (feed consumption: weight gain) during the five weeks of observation ducks were given the control treatment was 6.41/head (Table 3), and did not show any significant differences (P>0.05) with duck treatment B. the average of the value of FCR in ducks treatment C and D, respectively 17.32% and 15.44% (P<0.05) lower than the control.

Table 2. Supplementation of culture cellulolytic bacteria isolated from the rumen of buffalo into tofu-based rations on performance and carcass of growing male Bali duckling up to ten weeks old.

Variable Observed	Supplemented cellulolytic bacteria culture (%) in diets				SEM[4)]
	0.00	0.20	0.40	0.60	
Final body weight (g)	1174.62b[5)]	1196.15b	1328.91a	1306.47a	31.902
LWG (g/5 weeks) [1)]	659.30b	678.05b	812.81a	792.47a	29.714
FC (g/5 weeks) [2)]	4226.92a	4276.10a	4308.52a	4295.71a	65.804
FCR (feed/gains) [3)]	6.41a	6.31a	5.30b	5.42b	0.319
Carcass weight (g)	787.94b	812.78b	907.11a	893.76a	30.067
Carcass percentage (%)	67.08a	67.95a	68.26a	68.41a	0.647
Breast meat (% carcass weight)	11.15b	12.69a	13.24a	13.15a	0.375

Notes:

1. *Live weight gains*
2. *Feed consumption*
3. *Feed conversion ratio (feed consumption : live weight gains)*
4. *SEM :"Standard Error of Treatment Means"*
5. *Means with different superscripts within raws are significantly different (P<0.05)*

The average carcass weight of ducks were given a control did not show significant differences (P>0.05) with a carcass weight of ducks were treated B. But the duck carcass weight treatment C and D respectively: 15.12% and 13.43% (P<0.05) higher than the control. Mean percentage of duck breast meat controls showed no significant differences (P>0.05) with duck breast meat that are treatment B. But the percentage of breast meat ducks treatment C and D respectively 18.74% and 17.93% (P<0.05) higher than control

The average amount of abdominal fat ducks were given rations with addition of 0.20% cellulolytic rumen bacteria culture did not show any significant differences (P>0.05) with the controls. However, the addition of cellulolytic rumen bacteria culture in tofu based rations on the level of 0.40% (C) and 0.60% (D), respectively 16.67% and 18.60% (P<0.05) lower than the control.

Mean serum cholesterol levels in the blood of ducks which were 0.20% cellulolytic rumen bacteria culture in the basal diet did not show any significant differences (P>0.05) with the controls. However, the addition of cellulolytic rumen bacteria culture in the tofu-based ration at the level of 0.40% (C) and 0.60% (D) showed significant differences (P<0.05) respectively: 8.61% and 10.47% lower than the control.

Table 3. Supplementation of cellulolytic bacteria (isolated from the rumen of buffalo) in the tofu-based rations on slaughter weight, abdominal fat, serum cholesterol levels, and the levels of N-NH_3 in excreta of ducks up to tens weeks of age.

Variable Observed	Treatments[1)]				SEM[2)]
	A	B	C	D	
Abdominal-fat	2,58a	2,19b	2,15b	2,10b	0,083
Serum cholesterol (mg/dl)	177,05a	180,27a	161,81b	158,52b	4,029
N-NH_3 concentrations (m.Mol/liter)	12,593a	12,371a	11,291b	11,187b	0,375

Notes::

1. The tofu-based rations without supplementation culture cellulolytic bacteria isolated from the rumen of buffalo as a control (A); supplementing rations at 0.20% cellulolytic bacterial cultures (B); by supplementing 0.40% cellulolytic bacteria culture (C); and ration with supplementation of 0.60% cellulolytic bacteria culture (D);
2. SEM: Standard Error of Treatment Means
3. *Means with different superscripts within raws are significantly different (P<0.05)*

Mean levels of ammonia (N-NH_3) in excreta on ducks controls showed no significant differences (P>0.05) with the concentration of ammonia in the treatment of excreta B.

Supplementation of 0.40% (C) and 0.60% (D) cellulolytic bacterial cultures were isolated from the rumen of buffalo in the tofu-based rations, significantly different ($P<0.05$) can reduce levels of ammonia in the excreta in ducks, which is 10.34% and 11.16% lower than the control.

DISCUSSION

The results showed that supplementation of 0.40 to 0.60% of buffalo rumen cellulolytic bacterial culture into tofu-based rations (using tofu 30%) significantly increased ($P<0.05$) on the performance of ducks (final body weight, live weight gains, and feed efficiency). This is because the culture of cellulolytic rumen of buffalo that have passed the test as well as the probiotic agent and has cellulolytic activity or having the activity of CMC-ase (Bidura *et al*., 2014) in the digestive tract of ducks.

According to Piao *et al*. (l999), probiotic supplementation in the diet can significantly improve weight gain, utilization of nutrients, as well as nitrogen and phosphorus digestibility. The same thing was reported by Stanley *et al*. (l993), that the broilers were given probiotics 0.10% can significantly improve weight gain and feed efficiency. Feeding containing probiotics can improve the metabolism of nutrients in the digestive process (Nurhayati, 2008).

Han *et al.* (1999) suggest that supplementation of *Aspergillus oryzae* and *S.cerevisiae* in the basal ration at the level of 0.15% and 0.30% could increase the activity of amylolytic and proteolytic enzymes in the digestive tract of chicken, so as to improve metabolizabel energy and protein digestibility of the ration. Increased digestibility of protein and energy can have an impact on improving the feed conversion ratio and increasing the growth of ducks.

The response of probiotics in poultry was different effect, and it is highly influenced by the strain of bacteria used as probiotics, dosage or level of administration, the composition of the ration, feeding system, the form of rations, and interactions with other feed additive. As reported by Mahfudz (2006), that the use of fermented tofu at the level of 10% in the diet apparently not significantly affect the growth and carcass chicken, but at the level of 15-20% in the diet can markedly improve growth and carcass weight.

Apparently, the role of culture cellulolytic bacteria were isolated from the rumen of buffalo as a probiotic agent in the digestive tract of ducks effective at the level of 0.40 to 0.60% in the ration. Results of this study are supported by Wu *et al.* (2005) and Huang *et al.* (2004) that *Aspergillus xylanase* supplementation in wheat bran-based diet 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* (9×10^9 cfu) derived from baker's yeast in a basal ration of broiler chickens significantly increase the digestibility of dry matter, protein digestibility, and protein efficiency ratio.

Supplementation culture cellulolytic bacteria isolated from the rumen of buffalo can produce the enzymes amylase and protease, that its presence in the digestive tract of ducks will increase the activity of this enzyme and the breakdown of nutrients into a form that is simple and easily absorbed by the digestive tract (Mulyono *et al.,* 2009). Jaelani *et al.* (2008) reported that fermentation of feed material (palm kernel meal) with *Trichoderma reesei* can improve metabolizabel energy and crude protein of feed.

Extracellular peroxidase enzymes actively working on lygnolysis activity, thus breaking the bond lignocellulose and lignin fraction breaks down into CO_2.

Fermentation using microbes can improve the nutrient content and digestibility of feed (Arsyad *et al.,* 2001; Hong *et al*., 2004). Chen *et al*. (2005) reported that the addition of 0.20% probiotic complex (*L. acidophilus* and *S. cerivisae*) in the basal diet can markedly increase the digestibility of dry matter (Bidura *et al.,* 2012; Candrawati *et al*., 2014; Utama, 2011). 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).

Supplementation of 0.40 to 0.60% buffalo rumen cellulolytic bacterial culture into tofu-based rations can markedly increase carcass weight, carcass percentage, and the percentage of breast meat of ducks. This is due to the presence of probiotic microbes in the digestive tract of ducks can increase the activity of enzymes, absorption of nutrients, and increase retention of protein and energy in the body ducks. Reported Yi *et al*. (l996), that supplementation of probiotics into the diet significantly increased nitrogen retention in broiler chickens, the fermentation process will break down proteins into amino acids and carbohydrates into carbon dissolved protein necessary for the synthesis of the body.

Tang *et al*. (2007) suggest that increased consumption of protein and amino acid lysine in broiler chickens caused an increase in the amount of breast meat compared with the consumption of protein and lysine are lower. Diet containing high protein can increase in breast meat components (Al-Batshan and Hussein, 1999; Bidura, 2012).

Supplementation of 0.40 to 0.60% in the culture of cellulolytic rumen buffalo in tofu-based rations (using tofu 30%) significantly reduced the amount of abdominal fat and serum cholesterol levels in ducks. Fat consumed in the gut digested by pancreatic enzymes and emulsified by bile salts into micelles or chylomicrons. These micelles that are absorbed by the body as a source of energy and basic materials forming cholesterol, then deposited in the organs of the body as fat and cholesterol. Nurhayati (2008) reported that the use of feed fermented by *A.niger* at the level of 10-30% significantly reduced abdominal fat weight. Min (2006) reported that feeding fermented significantly reduced fat content and increase the percentage of meat in pigs.

Cellulolytic culture isolates were isolated from the rumen of buffalo as a source of probiotics in the diet significantly reduced the concentration of ammonia gas in the excreta of ducks. The use of probiotic microbes in poultry reported to suppress the activity of the enzyme urease and lower the amount of uric acid in the digestive tract of chicken, because uric acid has been utilized as a microbial protein. Decreased levels of N-NH_3 on the duck excreta, according to Yeo and Kim (l997) due to the ability of probiotics (*Lactobacillus cassei*) can suppress the activity of urease enzyme in the small intestine, so that the levels of organic gases in excreta decreased. Han *et al.* (1999) suggest that supplementation of *Aspergillus oryzae* and *S.cerevisiae* in the basal diet significantly increased the number of lactic acid bacteria (LAB) and decreased the number of bacteria *E.choli* and aerobic bacteria in the excreta.

Lactic acid bacteria survive in the digestive tract of poultry, and for this reason the number of bacteria that can cause *E.choli* and N-NH_3 levels in excreta decreased. Supplementation of probiotics into the diet significantly improves live weight gains and decreased levels of N-NH_3 in the feces of ducks (Chen *et al*., 2005; Roni *et al.,* 2014).

Puspani *et al.* (2014) reported that supplementation of yeast in the ration containing pollard improve performance and reduce the content of ammonia gas in the excreta of broiler chickens. The same thing was reported by Bidura *et al*. (2014), that supplementation of culture *Saccharomyces spp* isolated from feces of Bali cattle as much as 0.20% in the diet significantly increased performance and decreased levels of ammonia gas in broiler excreta.

CONCLUSION

From these results it can be concluded that 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_3 in excreta of duck.

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Corresponding author: Dr. Siti, N.W., Faculty of Animal Husbandry, Udayana University, Denpasar-Bali, Indonesia, Jl. PB. Soedirman, Denpasar-Bali
Email: siti_fapetunud@yahoo.com