Effect of Additional Probiotics of Cellulolytic Bacteria (Isolation from Buffalo Ruments) in Drinking Water on Egg Production Performance and Yolk Kolesterol in Laying Hens

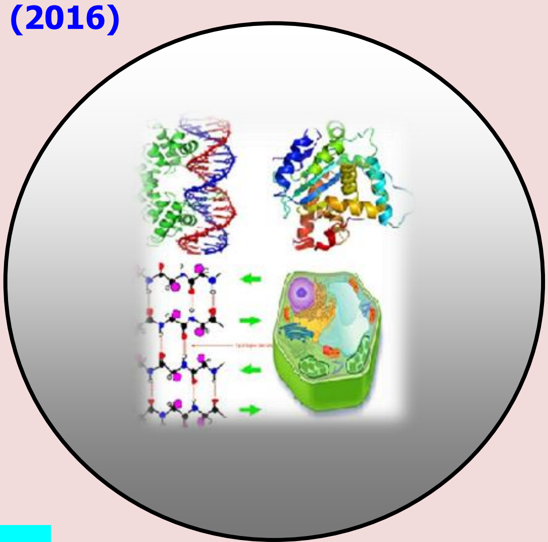
By

Desak Putu Mas Ari Candrawati, I.G.N.G. Bidura and D. A. Warmadewi

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 34 (2) 2017 Pages No. 878-884



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 34, No. 2: 878-884, 2017
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 34/02/779/2017

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

D.P.M.A. Candrawati
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 19/11/2017 **Revised: 01/12/2017** **Accepted: 02/12/2017**

Effect of Additional Probiotics of Cellulolytic Bacteria (Isolation from Buffalo Ruments) in Drinking Water on Egg Production Performance and Yolk Kolesterol in Laying Hens

Desak Putu Mas Ari Candrawati, I.G.N.G. Bidura and D. A. Warmadewi
Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia 80223
Jl. PB Soedirman, Denpasar-Bali, Indonesia 80223

ABSTRACT

This study aims to examine the effect of probiotic cellulolytic bacteria isolated from buffalo rumen in drinking water to egg production and egg cholesterol levels in Lohman Brown chickens 40-48 weeks old. A total of 120 chickens, 40 weeks old were randomized in a complete randomized design (RAL) with four treatments and 6 replications. The four treatments were chickens given drinking water without the addition of probiotics as control (A); drinking water with 0.20% probiotics (B); 0.40% probiotics, and 0.60% probiotics, respectively. The results showed that the addition of 0.20-0.60% probiotic bacteria cellulolitic isolated from buffalo rumen in drinking water, were significantly different (P<0.05) increased the feed consumption and drinking water, the number of eggs, egg weight, yolk color, eggshell thickness, and feed efficiency. In contrast, significantly (P<0.05) reduced both the fat and cholesterol content in egg yolks. It can be concluded that the addition of 0.20-0.60% probiotic cellulolytic bacteria isolated from buffalo rumen in drinking water can increase egg production of Lohmann Brown hens that are 40-48 weeks old and reduced both the fat and cholesterol content in egg yolk.
Keywords: Probiotics, Fat, Cholesterol and Eggs.

INTRODUCTION

Eggs become one of the favorite foods of many people because it has a delicious flavor, easy to digest and rich in nutritional value such as proteins, vitamins, minerals and also the fats the body needs. On the other hand, high levels of fat and cholesterol in products of animal origin consumed including eggs become a frightening specter for people because consuming excessive fat/cholesterol will affect health.

Therefore, it is very useful if it can lower cholesterol and fat in chicken eggs by utilizing probiotic biotechnology. Use of antibiotics to stimulate the growth of poultry has been banned in Europe and the United States (Ahmad, 2006). Nutritionists and livestock production are attracted to compounds that can act as antibiotic replacements. Probiotics are one approach that has the potential to replace antibiotics. Probiotics are living microorganisms that, when administered through the digestive tract, have a positive impact on health and host production. Probiotics are expected to increase the role of normal flora in the gastrointestinal tract to produce exogenous enzymes, such as amylases, proteases, and lipases that can increase endogenous enzyme activity to hydrolyze feed (Putra *et al.,* 2015).

Probiotic is a live microbial food supplements with various beneficiary effects on human health. There are more evidence for the use of probiotic as therapeutic substance for the treatment of different disorders like gastrointestinal infections, allergy, inflammatory bowel syndrome, pouchitis, diarrhea, colon cancer, etc., with dairy and non- dairy products. Probiotic not only beneficiary for humans, it is also used for animal health (Gowri *et al*., 2016) have been advised to be associated with improvement of lactose intolerance (Levri *et al*, 2005); prevention and treatment of viral, bacterial and antibiotic or radiotherapy induced diarrheas (Guandalini, 2006; Parvez *et al*., 2006); immunomodulation (Forsythe and Bienenstock, 2010); antimutagenic (Chalova, 2008) and anticarcinogenic effects (Liong, 2008) and even blood cholesterol decrease (Ooi and Liong, 2010).

Some preliminary research results on the use of probiotics in diets was able to improve performance, fiber digestibility, and lower the amount of fat or cholesterol in the body of poultry. Bidura *et al.* (2012) reported that the use of *Saccharomyces sp* probiotics isolated from yeast in rations containing 15% rice bran can increase body weight gains, as well as decrease the amount of abdominal fat and cholesterol. Probiotic supplementation can significantly increase the growth and decrease serum cholesterol in poultry, improve feed efficiency, and lower serum cholesterol levels (Bidura *et al*., 2014; Candrawati *et al*., 2014; Astuti *et al.,* 2017; Ristiani *et al*., 2017). While, Puspani *et al*. (2016) reported that supplementation of 0.2-0.6% isolate of B7 cellulolytic bacteria isolated from buffalo rumen in ration can increase body weight gains and feed efficiency in ducklings aged 2-8 weeks. From the above description, it is necessary to conduct research using cellulolytic probiotics isolated from buffalo rumen in drinking water as an effort to reduce fat content and cholesterol levels in eggs without adversely affecting its production performance of laying hens.

MATERIAL AND METHODS

Experimental birds and design of the experiment: A total of 120 chickens, 40 weeks old were randomized in a complete randomized design (RAL) with four treatments and 6 replications. The four treatments were chickens given drinking water without the addition of probiotics as control (A); drinking water with 0.20% probiotics (B); 0.40% probiotics, and 0.60% probiotics, respectively. All chickens were given commercial feed specific for laying hens containing 2.750 kcal/kg of Metabolizable Energy (ME); 18% of CP; 3.5% of Ca; and available phosphor of 0.45%. The birds were were housed in pens whose floors were covered with wood shavings. Each cage was 120×100×50 cm and was equipped with a feeding and drinking trough as well as lamps for heating and lighting. Feed and water were provided *ad libitum.* The necessary routine management, vaccinations and medications were provided. Food and drinking liquid were given *ad libitum*. The individual hens were weighted weekly, and food consumption and egg production was recorded daily. Other routine poultry management practices were maintained. The feeding trial lasted for 48 days.

Performance, egg quality metrics, and laboratory analysis: Eggs were collected and labeled on a daily basis at 08.00 h and 14.00 h throughout the experimental period. The percent egg production was calculated. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk cholesterol content was analyzed for two consecutive weeks. Cholesterol levels were analyzed following the Liberman-Burchard methods (1980).

Statistical analysis: All data were analyzed with ANOVA to determine the differences among treatments. If differences were found, then further analysis was performed withDuncan's multiple range test.

RESULTS

The results study shows that supplementation of 0.2% (treatment B); 0.40% (treatment C); and 0.60% (treatment D), probiotic cellulolytic bacteria isolated from rumen of buffalo in drinking water significantly increased (P<0.05) the average number of eggs produced, the efficiency of feed consumption, water consumption, egg weight per head (g/head), the average total egg weight as well as the average hen-day production. In addition, the results show that an additional 0.20-0.60% of probiotic cellulolytic bacteria in drinking water resulted in a significant (P<0.05) decrease in levels both of cholesterol and fat in the yolk of birds.

The average feed consumption for eight weeks in chickens given drinking water without the addition of probiotic bacteria cellulolitik (treatment A) was 7345.8 g/head, while the average feed consumption in chicken treatment B; C; and D; were 4.51%, 4.36%; and 4.83%; respectively (P<0.05) higher than those treated with A.

The results showed that the average consumption of drinking water in chickens given drinking water without the addition of probiotic cellulolytic bacteria or control were: 21.73 liters/head/8 weeks, while the average consumption of drinking water in chicken treated B; C; and D; are: 9,20%, 13,85% and 12,37%, respectively (P <0,05) higher than control.

Table 1. The effect of probiotic cellulolytic bacteria isolated from rumen of buffalo added in drinking water and administered to 40-48 weeks aged of egg laying hens to the egg production and yolk cholesterol level.

Variables	Treatments[1)]				SEM[2)]
	A	B	C	D	
Feed Consumption (g/head/days)	7345,8 $^{b3)}$	7676,7^{a}	7665,5^{a}	7700,2^{a}	37,93
Water onsumption (g/head/days)	21,73$^{b2)}$	23,73^{a}	24,74^{a}	24,42^{a}	0,41
The numer of eggs (egg/56 days)	42,12^{b}	44,43^{a}	44,49^{a}	44,39^{a}	0,28
Total egg weight (g/head/56 days)	2360,9^{b}	2639,3^{a}	2650,3^{a}	2641,4^{a}	25,23
Yolk color (1-15)	7,93^{b}	8,42^{a}	8,61^{a}	8,56^{a}	0,14
Shell thickness (mm)	0,40^{b}	0.48^{a}	0.47^{a}	0,46^{a}	0,02
Feed conversion ratio (feed consumption: total egg weight)	3,11^{b}	2,91^{a}	2,90^{a}	2,92^{a}	0,04
Yolk cholesterol (mg/dl)	176,23^{b}	160,35^{a}	159,29^{a}	160,64^{a}	3,69
Egg fat (% DM)	29,54^{b}	28,05^{a}	27,91^{a}	27,95^{a}	0,26

Notes:
1. A: drinking water without probiotic cellulolytic bacteria as control; (B): drinking water with 0.20% probiotic cellulolytic bacteria; (C): drinking water with 0.40% probiotic cellulolytic bacteria, and (D): drinking water with 0.60% probiotic cellulolytic bacteria, respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

The average egg production in the treated chicken A was 42.12 grains/head/8 weeks, while the average egg production for eight weeks in treated chickens B, C, and D, were: 5.48%; 5.62%; and 5.38%, respectively (P<0.05) were higher than those treated with A. The total egg weight in the treated chicken A was: 2360.9 g/head, whereas the total egg weight in the treated chickens B, C, and D, were: 11.79%; 12.25; and 11.88%, respectively (P<0.05) were higher than those treated with A.

The color of egg yolk in the treated chicken A was: 7.93, while the yolk color in the treated chickens B, C, and D, each significantly (P<0.05) was higher than treated chickens A. The thickness of the shel eggs of the treated chickens was: 0.40 mm, while the average thickness of egg shel in treated chickens B, C, and D, were: 20%; 17.5%; and 15.00%; respectively (P<0.05) higher than those treated with A.

The average value of FCR (feed consumption: total egg weight) in treated chickens A was: 3.11/birds and the average FCR value in chickens treated with B, C, and D, were: 6.43%; 6.75%, and 6.10%, respectively (P<0.05) were significantly lower than treated chickens A.

The cholesterol content of eggs in the treated chicken A was 176,23 mg/dl, while the treated chicken B, C, and D, were 9.01%; 9.61% and 8.84%, respectively significantly lower (P<0.05) than those treated with A. The content of egg fat in the treated chicken A was 29.54% DM, while the egg fat content in chickens treated with B, C, and D, was 5.04%; 5.51%; and 5.38%, respectively significantly lower (P<0.05) than control (A).

DISCUSSION

Supplementation of 0.20-0.60% probiotic bacteria cellulolytic isolated from rumen buffalo in drinking water, can increase feed and drink consumption compared to control. The increasing consumption of drinking water in the treatment of B, C, and D is caused by the high consumption of feed in treated chickens B, C, and D. The chicken generally consume drinking water two times greater than the amount of feed consumed, because drinking water serves as a solvent and a means of transporting nutrients to be dispersed throughout the body. While the high consumption of feed in treated chickens B, C, and D, due to the cellulolytic bacteria cultures isolated from buffalo rumen that has passed the test as a probiotic agent and increase activity of CMC-ase. The results of this study are consistent with Ristiani *et al*. (2017) found that probiotic supplementation *Saccharomyces spp* in ration at level 0.20-0.40% real can increase consumption of feed and drinking water in broiler chickens. In contrast, Bidura *et al.* (2016), and Bidura *et al.* (2012) reported that probiotic supplementation in ration had no significant effect on feed and drink consumption. The same thing was reported by Bidura *et al.* (2016) that supplementation of *Saccharomyces spp*.Sb-6 probiotic at level 0.20-0.60% in ration has no significant effect on ration consumption, but significantly increase egg weight and egg production in chicken.

Table 1 shows that egg production and egg weight in treated chickens B, C, and D were higher than treated chickens A. This is due to the culture of cellulolytic bacteria isolated from the buffalo rumen that has passed the test as a probiotic agent, can increase the consumption of feed and digestibility of nutrients, such as carbohydrates, proteins, and fats into simple compounds, making it more easily absorbed by chickens. This condition will in turn increase egg production and egg weight. This is in accordance with the opinion of Bidura *et al*. (2016) that probiotic supplementation in the ration at level 0.20-0.60% can significantly increase hen-day production and total egg weight in laying hens.The color of the egg yolk in the treated chickens B, C and D, was significantly (P<0.05) higher than the treated chicken A (control). This is caused by the cellulolytic bacteria cultures isolated from buffalo rumen that have passed the test as probiotic agents cause the digestion process to be better so that it will increase the consumption of feed. The color of the egg yolk is greatly influenced by the beta-carotin content in the rations that are eaten. So, the increased consumption of rations, will cause the higher beta-carotin is absorbed, so that will affect the color of egg yolks. Increased feed consumption and nutrient digestibility, including calcium minerals, can cause increased shell thickness. This is in accordance with research Bidura *et al*. (2016), that probiotic supplementation *Saccharomyces spp*. in the ration at the level of 0.20-0.60% can significantly increase the shel thickness and total egg weight in laying hens. In Table 1 it is shown that the layers of Chicken group that received treatment of B, C and D, were more efficient in using ration to increase egg production, especially chickens got treatment C.

It is caused by the culture of cellulolytic bacteria in drinking water that can improve digestibility nutrient to be used to increase egg production. This opinion is in agreement with Abdel-Hafeez *et al*. (2017) and Ristiani *et al.* (2017) who reported that probiotic supplementation in rations could improve feed efficiency in broiler and in laying hens.

Cholesterol concentration in egg yolk, was influenced by cholesterol levels ready to be distributed from the digestive tract. The existence of cellulolytic bacteria cultures isolated from buffalo rumen, was able to produce an enzyme called the enzyme Bile Salt Hydrolase (BSH), an enzyme that can deconjugate bile salts. Furthermore, bile salts will be excreted through the feces, so the amount of bile acid returning to the liver will be reduced. To balance the concentration of bile salts in the liver, the body will take blood cholesterol as its precursor material. This process will eventually be able to lower cholesterol levels in the blood. According to Ngatirah *et al*. (2000) which states that there are several mechanisms of probiotic bacteria in lowering blood cholesterol. The suspected mechanism is the ability of probiotics that can degrade cholesterol to coprostanol. Koprostanol is a sterol that can not be absorbed by the intestine, so koprostanol along with other cholesterol will be removed with the feces. Ristiani *et al.* (2017) reported that supplementation of 0.20-0.40% of probiotics *Saccharomyces spp* in rations, can lower cholesterol content in meat and lower abdominal fat. Also reported by Bidura *et al.* (2016), that probiotic supplementation in rations may decrease cholesterol content in serum and eggs in laying hens.

Supplementation of probiotic cellulolytic bacteria in drinking water at the level of 0.20-0.6% were significantly decrease the fat content in eggs compared with controls. The fat content of chicken eggs treated with C was the lowest. The low fat content is due to the culture of cellulolytic bacteria isolated from buffalo rumen that has passed the test and has a role as probiotic agent.

According to Santoso *et al*. (1995), the provision of probiotics in the diet can reduce fat, because probiotics can effectively decrease the activity of acetyl coenzyme A carboxylase, an enzyme that plays a role in the rate of fatty acid synthesis. Decreased levels of fat in the digestive tract of poultry can cause fat to be brought into the ovaries to be synthesized in the yolk will be reduced. Kalavathy *et al.* (2003) reported that Lactobacillus culture supplementation at 0.10% in ration significantly reduced serum triglyceride concentrations. Homma and Shinohara (2004) also reported that the inclusion of *Bacillus cereus toyoi* in the male quail ration as a source of probiotics, significantly reduced the accumulation of fat in the abdomen and viscera. Saleh *et al*. (2011) reported that 0.01% *Aspergillus awamori* supplementation or 0.05% *Aspergillus niger* in the ration significantly reduced the fat content in the body, because probiotics can inhibit lipid biosynthesis (Yamamoto *et al*., 2007; Mansoub and Kafshnochi, 2010), as well as to increase the catabolism of fatty acids in the body (Homma and Shinohara, 2004). Reported by Ooi and Liong (2010) that probiotics in the digestive tract can inhibit the absorption of cholesterol so that synthesed cholesterol in the body becomes decreased. In contrast, Abdel-Hafeez *et al.* (2017) reported that probiotics in the ration had no significant effect on total protein and cholesterol content in blood serum, but significantly reduced the fat content in carcasses. The results of Puspani *et al.* (2016) reported that supplementation of probiotic bacteria Cellulolitik B-7 at level 0.20-0.60% isolated from buffalo rumen, can significantly reduce the cholesterol content in serum and abdominal fat in the body of the ducks.

CONCLUSION

It can be concluded that giving of 0.20-0.60% probiotic cellulolytic bacteria isolated from buffalo rumen in drinking water can improve the performance of egg production and and may decrease both fat and yolk cholesterol in laying hens up to fourty weeks of age.

ACKNOWLEDGEMENTS

The authors would like to thank to staff of laboratory attendants at the Nutrition Laboratory, Udayana University for their assistance in chemical analysis of the samples. We also would like to thank the Head of Research and Public Service Department and Dean of Faculty of Animal Science, Udayana University for their support during this study including research funding.

REFERENCES

Abdel-Hafeez, H.M., Elham S. E. Saleh, Samar S. Tawfeek, Ibrahim M. I. Youssef, and Asmaa S. A. Abdel-Daim. **2017**. Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian-Australas J Anim Sci 30:672-682.

Ahmad, I., 2006. Effect of probiotics on broilers performance. Int. Poult. Sci., 5: 593-597.

Astuti, S. Umniyati, A. Rahmawati, and E. Yulianti. 2017. The effect of acid *Lactic bacteria* Ast 6 Streptococcus thermophilus to broilers abdominal fat. J. Biol. Chem. Research Vol 34 (1): 62-67.

Bidura, I.G.N.G., I. B. Sudana, I. P. Suyadnya, I. G. Mahardika, I.G.L. Oka, I. B. Gaga Partama, and I.G.A.I. Aryani. 2012. The implementation of *Saccharomyces spp.n-2* isolate culture (isolation from traditional yeast culture) for improving feed quality and performance of male Bali duckling. Agricultural Science Research Journal Vol. 2 (9): 486-492.

Bidura, IGNG., N.W. Siti and I. A. Putri Utami. 2014. Isolation of cellulolytic bacteria from rumen liquid of buffalo both as a probiotics properties and has CMC-ase activity to improve nutrient quality of soybean distillery by-product as feed. International Journal of Pure & Applied Bioscience September Vol. 2 (5): 10-18.

Bidura, IGNG., I.B.G. Partama, D.K.H. Putra and U. Santoso. 2016. Implementation on diet of probiotic *Saccharomyces spp*.SB-6 isolated from colon of Bali cattle on egg production and egg cholesterol concentration of Lohmann brown laying hens. Int. J. Curr. Microbiol. App. Sci. Volume 5 (4): 793-802.

Candrawati, DPMA., DA. Warmadewi and IGNG. Bidura. 2014. Implementation of *Saccharomyces spp*.S-7 isolate (Isolated from manure of Bali cattle) as a probiotics agent in diets on performance, blood serum cholesterol, and ammonia-N concentration of broiler excreta. International Journal of Research Studies in Biosciences Vol. 2 (8): 6-16.

Chalova, V.I., Lingbeck, J.M., Kwon, Y.M., Ricke, S.C. 2008. Extracellular antimutagenic activities of selected probiotic *Bifidobacterium* and *Lactobacillus* spp. as a function of growth phase. *J. Environ. Sci. Health*, 43(2): 193–8.

Ezema, C. and D. C. Eze. 2015. Probiotic effect of yeast (*Saccharomyces cerevisiae*) on hen-day egg performance, serum and egg cholesterol in laying chickens. Pakistan Journal of Nutrition 14 (1): 44-46.

Forsythe, P., Bienenstock, J. 2010. Immunomodulation by commensal and probiotic bacteria. *Immunol. Invest,* 39(4–5): 429–48.

Gowri, R.S., P. Meenambigai, P. Prabhavathi, P. Raja Rajeswari and L. Arul Yesudoss. 2016. Probiotics and its Effects on Human Health-A Review.2016. *International Journal of Current Microbiology and Applied Sciences* Volume 5 (4): 384-392.

Guandalini, S. 2006. Probiotics for children: use in diarrhea. *J. Clin. Gastroenterol.,* 40(3): 244–8.

Homma, H. and T. Shinohara. 2004. Effects of probiotic Bacillus cereus toyoi on abdominal fat accumulation in the Japanese quail (*Coturnix japonica*). Anim. Sci. J. 75:37-41.

Mansoub, NH, and Kafshnochi M. 2010. The effects of probiotics and food restriction on relative growth and serum cholesterol and triglycerides contents in broiler chickens. Global Vet 5: 307-11.

Kalavathy, R., N. Abdullah, S. Jalaludin, and Y. W. Ho. 2003. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br. Poult. Sci. 44:139-144.

Levri, K.M., Ketvertis, K., Deramo, M., Merenstein, J.H., D'Amico, F. 2005. Do probiotics reduce adult lactose intolerance. A systematic review. *J. Fam. Pract.,* 54(7): 613–20.

Lieberman, A. and R. Burchard, 1980. Enzymatic method to determined cholesterol. Engl. J. Med., 271: 915-924.

Ngatirah, Harmayani E, Rahayu ES, Tyas U. 2000. Seleksi bakteri asam laktat agensia probiotik yang berpotensi menurunkan kolesterol. Seminar Nasional Industri Pangan. PATPI. Surabaya, 10-11 Oktober 2000.

Ooi, L.G., and Liong, M.T. 2010. Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. *Int. J. Mol. Sci.,* 11(6): 2499–522.

Parvez, S., Malik, K.A., Ah Kang, S., Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. *J. App. Microbiol.,* 100(6): 1171–85.

Puphan, K., P. Sornplang and S. Uriyapongson. 2013. Cultivation of *Lactobacillus* sp. and Production of Probiotic Powder as Animal Feed Additive. Pakistan Journal of Nutrition 12 (6): 567-570.

Puspani, E., DPMA. Candrawati, and IGNG. Bidura. 2016. Implementation of probiotics cellulolitic B-7 bacteria (isolation from buffalo rumen) into ration on the performance, abdominal fat and serum cholesterol of duck. Int. J. Curr. Microbiol. App. Sci. Volume 5 (11): 432-441.

Putra, A.N., N.B.P. Utomo and Widanarni. 2015. Growth Performance of Tilapia (*Oreochromis niloticus*) Fed with Probiotic, Prebiotic and Synbiotic in Diet. Pakistan Journal of Nutrition 14 (5): 263-268.

Ristiani, N.M., I G.N.G. Bidura and Dewi Ayu Warmadewi. 2017. The Effect of *Saccharomyces spp*.Gb-9 (Isolated from Colon of Native Chicken) on the Growth Performance and Meat Cholesterol Level in Broilers. J. Biol. Chem. Research. Vol. 34, No. 1: 80-88.

Saleh, A. A., Z. Eid, and K. Hayashi. 2011. Effects of feeding *Aspergillus awamori* and *Aspergillus niger* on growth performance and meat quality in broiler chickens. J. Poult. Sci. 48: 201-206.

Santoso, U., K. Tanaka, and S. Ohtania. 1995. Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. Br. J. Nutr. 74:523-529.

Yamamoto, M., F. Saleh, M. Tahir, A. Ohtsuka, and K. Hayashi. 2007. The effect of Koji-fed (fermented distillery byproduct) on the growth performance and nutrient metabolizability in broiler. J. Poult. Sci. 44:291-296.

Corresponding author: Dr. Desak Putu Mas Ari Candrawati, Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia 80223, Jl. PB Soedirman, Denpasar-Bali, Indonesia 80223
Email: dsk_candrawati@unud.ac.id