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THE EFFECT OF YEAST CULTURE (*SACCHAROMYCES SPP*.) SUPPLEMENTATION IN DIETS ON FEED EFFICIENCY AND YOLK CHOLESTEROL LEVELS IN LOHMANN BROWN LAYING HENS

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ABSTRACT

This study was planned to examine the effect of inclussion of yeast culture (YC) in the ration as a probiotic on feed efficiency and yolk cholesterol levels in Lohmann Brown laying hens. This study used as many as two hundred and forty Lohmann Brown laying hens up to 70 weeks old in a completely randomized design with 4 kinds of treatments, namely: the diets (CP: 17% and ME: 2750 kcal/kg) were fortified with levels of probiotik YC at 0.0%, 0.1%, 0.2%, and 0.30% to prepare diets Y0, Y1, Y2, and Y3, respectively. The results showed that supplementation from 0.20 to 0.30% of yeast culture in the ration, was significant ($P<0.05$) can increase total egg weight, egg production, feed efficiency, egg shell thickness and egg yolk color. Supplementation of yeast culture in the ration apparently had no significant effect ($P>0.05$) on feed consumption and final body weight. Dietary inclusion of 0.20-0.30% YC significantly ($P<0.05$) reduced the levels of yolk cholesterol in the hens of all treated groups when compared to those of control group. It was concluded that supplementation from 0.20 to 0.30% Yeast culture in the ration could increase egg production, feed efficiency, eggshell thickness and egg yolk color of Lohmann Brown hens, and reduce yolk cholesterol content.

KEYWORDS: Probiotics, eggshell thickness, yolk color, cholesterol.

INTRODUCTION

The most important thing in laying hens is to improve feed efficiency. Feed efficiency can be measured from the value of the feed conversion ratio (FCR), which is the ratio between the amount of feed consumed with the weight of eggs produced in the same unit of time (Bidura *et al*., 2019). The lower FCR value means higher feed efficiency and vice versa. In the rearing system of laying poultry, FCR is highly influenced by the quality of the ration consumed, and one of them is the content of protein, energy, and crude fiber, and Antibiotic Growth Promotors (AGPs). One effort to improve feed efficiency or reduce FCR values and improve the appearance of laying poultry production is by adding various feed additives, such as enzymes and AGPs. But, the use of AGPs in Indonesia has been banned since January 2018 (Minister of Agriculture, Indonesia, 2017), because the residues have an adverse effect on consumers, so it is necessary to look for other safe feed additives, such as probiotics (Smirnov *et al.*, 2005).

Probiotic microbes can change the microbial ecosystem in the digestive tract of poultry into more useful microbes (Zurmiati *et al*., 2014; Mountzouris *et al.*, 2010), can improve feed digestibility (Zhen *et al*., 2019; Liu *et al*., 2018; Bidura *et al*., 14a,b; 2019; Mikulski *et al*., 2012), can improve plasma immunoglobulins and cecal microflora composition in broilers (Fathi *et al*., 2017). The probiotic inclusion levels in broiler diets can improve growth performance, nutrient digestibility and increase antioxidant enzyme activities in pigs (Mountzouris *et al*., 2010; Lei *et al*., 2018). Probiotics can increase the absorption of Ca and P minerals, thereby increasing egg shell thickness (Zhen *et al*., 2019). In addition, it also produces natural antibiotics, so that it influences the health and performance of the host (Zurmiati *et al*., 2014; 2017; Bidura *et al*., 2019). The use of probiotics has been widely applied to chickens, but the responses obtained are very different (Bidura *et al*., 2012; 2014a,b; 2016; Bidura, 2019; Candrawati *et al*., 2014; Lei *et al*., 2013; Mikulski *et al*., 2012; Mountzouris *et al*., 2010; Waqas *et al*., 2019). Probiotic bacteria in the digestive tract of chickens, can inhibit the growth and activity of pathogenic microorganisms that can convert uric acid into ammonia by using the urid acid as a nutrient (Riza *et al*., 2018). Decreasing ammonia gas levels in the cage will increase the comfort of the chicken in utilizing nutrients, so that feed efficiency can be increased (Carvalho *et al*., 2019).

Research on the use of probiotics as feed additives still needs to be improved, especially in rice bran-based rations, in order to obtain efficient and practical production techniques that can be applied, so as to be able to provide an economic impact on the people's scale livestock business. This study aims to examine the supplementation of *Saccharomyces spp*. probiotics in rations on feed efficiency and cholesterol levels in chicken egg yolk.

MATERIAL AND METHODS

Animal treatments and experimental design

Two hundred and fourty 70-w-old healthy Lohmann Brown hens were used in a completely randomized design experiment. The diets (CP 17% and ME 2750 kcal/Kg) were fortified with levels of probiotik YC at 0.0%, 0.1%, 0.2%, and 0.30% to prepare diets Y0, Y1, Y2, and Y3, respectively. All diets were mas form, and the diets were formulated to meet the nutrient requirements for poultry hens (Scott *et al*., 1982) for eight weeks of experiment. The ingredients and chemical compositions of experimental diets are shown in Table 1.

Table 1: The ingredient and calculated nutrient content of the feed of Lohmann Brown laying hens up to 70 weeks old.

	Treatments			
	Y0	**Y1**	**Y2**	**Y3**
Ingredients (%):				
Yellow corn	56	56	56	56
Rice bran	19	18,9	18,8	18,7
Soybean	4,1	4,1	4,1	4,1
Fish meal	14,5	14,5	14,5	14,5
NaCl	0,4	0,4	0,4	0,4
Mineral-B12**)	6	6	6	6
Yeast culture (*Sacharomyces spp*)	0	0,10	0,20	0,30
Total	100	100	100	100
*Chemical composition**) *:*				
Metabolizable energy (Kcal/kg)	2751	2750	2748	2750
Crude protein (%)	17,06	17,05	17,04	17,03
Ether extract (%)	6,7	6,68	6,67	6,66
Crude fibre (%)	3,75	3,74	3,73	3,71
Calcium (%)	4,1	4,1	4,1	4,1
Posphor (%)	1,51	1,51	1,51	1,51
Arginine (%)	1,24	1,24	1,24	1,24
Cystine (%)	0,33	0,33	0,33	0,33
Liusyne (%)	1,67	1,67	1,67	1,67
Methionine (%)	0,44	0,44	0,44	0,44
Trypthophan (%)	0,18	0,18	0,18	0,18

Note
* Based on calculation according to Scott *et al.* (l982)
**Calsium 49; phosphor 14; iron 40000 mg; manganese 27500 mg; iron 40 mg; mg 27.500 mg; zinccum 25 mg; vit b12 4.50 mg dan vit d3 500000 iu. PT Eka Farma. Deptan RI No. D 8109127 FTS.

Probiotics prepared

Yeast culture used as a source of probiotics used in this study was *Saccharomyces spp*. isolated from "tape" yeast. *Sacharomyces spp*. isolates have passed the test and have been considered as potential probiotics according to our previous research (Bidura *et al*., 2012). *Saccharomyces spp.* Isolates that have passed were approved from the bile salt test and in vitro tests in the digestive tract of poultry, capable of carrying out de-conjugation of bile acids, and both as probiotic agents and have CMC-activity as well. Probiotics were prepared at the Nutrition and Technology Laboratory at the Faculty of Animal Sciences, Udayana University. Every 1 g of culture contained *Saccharomyces spp.* a total of 10^6 CFU/g (Bidura *et al*., 2012).

Quality of eggs and yolk cholesterol: The research house was given continuous lighting and access to feed and water was provided during the experiment. Birds were weighed at the start of the experiment (70 weeks old) and the end of the experiment (80 weeks old). Eggs are collected daily and egg production is calculated. The individual egg weight recorded was then used to calculate the average egg weight for all trial periods. The total egg mass is calculated by multiplying the egg weight by egg production. Egg quality parameters were measured using a multi-egg tester. Feed intake is measured based on the cage (chicken) every week. Daily feed intake per bird was calculated based on the total intake of animal feed for the entire trial period. Feed conversion ratio (FCR) or feed efficiency (kg of feed/kg of egg mass), egg production, egg weight, and feed consumption, are calculated weekly. Egg yolk samples were analyzed to look for dry matter (DM) content by drying the sample at 105^0C for 24 hours in a forced air oven. Total cholesterol is determined in fat separated by extraction from egg yolks with a mixture of chloroform and methanol (2: 1 vol: vol). Cholesterol is separated from fat after saponification with KOH. Yolk cholesterol is calculated and expressed in milli grams/gram of egg yolk. (Bidura *et al*., 2019).

Statistical analysis: Data was analyzed by one way ANOVA Duncan's multiple range test was used to determine differences among treatment mean values (p<0.05).

RESULTS

The results of the study are presented in Table 2. Supplementation of YC probiotics in the ration at the level of 0.1-0.3% was found to have no significant effect (P>0.05) on final body weight and feed consumption. The number of chicken eggs during 10 weeks of observation in chicken fed rations without supplementation of probiotic YC as a control (Yo) was 68.26 eggs/head/10 weeks (Table 2). The number of eggs in chicken fed rations with supplementation of 0.10% YC (Y1) apparently had no significant effect (P>0.05) compared to controls (Y0). Supplementation of 0.20%YC (Y2) and 0.30% YC (Y3) probiotic in the ration, significantly (P<0.05) can increase the number of eggs, were: 6.40% and 6.83% higher compared to the control (Y0).

The average egg weight of Group Y0 chickens was 66.52 g/egg (Table 2) and showed no significant difference (P>0.05) with Group Y1. The average egg weight of chicken Group Y2 and Y3, were: 3.83% and 4.66% significantly (P<0.05) higher than controls. Likewise, the egg weight per week in Group Y2 and Y3 chickens was: 10.48% and 11.80% significantly higher (P<0.05) than controls (Group Y0 chickens).

The average FCR value (feed consumption: egg weight) for 10 weeks of observation in Group Y0 chickens was 2.84/bird (Table 2). The mean FCR in chicken Y1 group was 3.87% significantly (P<0.05) lower than the control (Y0). The average FCR values in chicken Y2 and Y3 groups were: 4.93% and 5.63% significantly (P<0.05) lower than controls.

Supplementation of 0.1-0.30% probiotic yeast culture isolates of "Tape" yeast in the ration apparently had no significant effect on ration consumption. The results of this study are the same as those of Bidura *et al*. (2016); Bidura *et al*. (2012); and Bidura (2019) reported that supplementation of probiotics in the ration did not significantly affect feed and drinking water consumption. The same thing was reported by Bidura *et al.* (2016) that supplementation of probiotic *Saccharomyces spp*.Sb-6 at the level of 0.20-0.60% in the ration had no significant effect on feed consumption, but significantly increased egg weight and chicken egg production. Contrary to research by Ristiani *et al*. (2017) who found that supplementation of *Saccharomyces spp*. in the ration at the level of 0.20-0.40% markedly increased consumption of feed and drinking water in broilers.

Table 2: Effect of *Saccharomyces spp*. probiotic supplementation in ration on egg production and feed efficiency in laying hens Lohmann Brown aged 70-80 weeks.

Variabel	Groups[1]				SEM[2]
	Y0	Y1	Y2	Y3	
Initial body weight (g)	1858.05a	1846.37a	1862.61a	1850.72a	32.83
Final body weight (g)	1864.63a	1859.52a	1865.95a	1866.74a	30.27
Feed consumption (g/head/weeks)	1126.51a	1098.85a	1183.92a	1190.63a	18.082
Egg numbers (egg/10 weeks)	68.26b[3)]	68.84b	72.63a	72.92a	0.508
Egg weight (g/egg)	66.52a	66.71a	69.07b	69.62b	0.275
Total egg weight (g/weeks)	397.31b	401.83b	438.95a	444.21a	3.084
FCR (feed consumption:egg weight)	2.84a	2.73b	2.70b	2.68b	0.032

Note

1. Y0): basal diet (control); Y1): basal diet+0.10% YC; Y2): basal diet+0.20% YC*;* and Y3): basal diet+0.30% YC, respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

Supplementation from 0.20 to 0.30% YC as a source of probiotics in the ration significantly increased egg production and egg weight. This is due to the presence of yeast *Saccharomyces spp.* as a probiotic in the digestive tract of chickens that can increase the digestibility of nutrients, such as carbohydrates, proteins, and fats into simple compounds, so that they are easily absorbed. This condition in turn can increase egg production and egg weight. This is consistent with the opinion of Bidura *et* *al.* (2016) that supplementation of *Saccharomyces spp*. in the ration at the level of 0.20 to 0.60% can significantly increase hen-day production and total egg weight in laying hens.

FCR values in chicken groups that received rations with supplementation of 0.10-0.30% YC were significantly lower than in control chickens (without supplementation). This means that chickens that receive rations with probiotic supplementation are more efficient at utilizing rations to increase egg production. This is due to the fact that YC culture as a probiotic can increase feed digestibility and is used to increase egg production. This opinion is the same as research by Abdel-Hafeez *et al.* (2017); Bidura (2019); and Ristiani *et al*. (2017) who reported that supplementation of probiotics in the ration could improve feed efficiency in broilers and feed efficiency in laying hens. Bidura *et al*. (2012) reported that administration of *S. cerevisiae* in feed can increase the digestibility of protein and crude fiber components, such as cellulose and hemicellulose, because the fiber fraction has been degraded in the form of simple monosaccharide compounds. Bidura and Siti (2017) reported that supplementation of probiotic *Saccharomyces spp.* in rations at the could increase the metabolic energy and protein digestibility. Increased protein digestibility and metabolic energy can have an impact on increasing feed efficiency and improving performance. Also reported by Liu *et al.* (2018) that the addition of probiotics in pig rations, significantly improved the digestion of feed. Rafique *et al.* (2020) reported that supplementation of *Saccharomyces cerevisiae* at a diet rate of 1.5 g/kg in feed, could significantly increase feed consumption, weight gain, efficiency of the digestive system, and feed efficiency which was most beneficial compared to control chickens.

YC supplementation in the ration at the level of 0.1-0.3% was significantly ($P<0.05$) on egg yolk color, eggshell thickness, and egg yolk cholesterol level (Table 3). The yolk color value of chicken fed rations without YC supplementation as a control (Yo) was 7.02 (Table 3). Egg yolk color in chicken fed rations with supplementation of 0.10% probiotic YC (Y1) did not show any significant difference ($P>0.05$) compared to controls (Y0). Supplementation of 0.20% YC (Y2) and 0.30% YC (Y3) in the ration, significantly ($P<0.05$) can increase the color of the yolk, namely: 27.07% and 32.19% higher than the control (Y0).

Table 3: Effect of Yeast culture supplementation in the ration on egg yolk color, eggshell thickness, and egg yolk cholesterol levels in laying hens Lohmann Brown aged 70-80 weeks.

Variables	Groups[1]				SEM[2]
	Y0	Y1	Y2	Y3	
Yolk color (1-15)	7.02b[3]	7.51b	8.92a	9.28a	0,407
Eggshell thickness (mm)	0.395b	0.398b	0.425a	0.437a	0.0105
Egg yolk cholesterol (mg/g)	7.75a	7.69a	5.75b	5.92b	0.407

Note

1. Y0): basal diet (control); Y1): basal diet+0.10% *YC*; Y2): basal diet+0.20% *YC;* and Y3): basal diet+0.30% *YC*, respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different ($P<0.05$)

The average eggshell thickness in Group Y0 chickens was 0.395 mm (Table 3) and did not show any significant difference ($P>0.05$) with eggshell thickness in Group Y1 chickens. The average eggshell thickness in chicken Group Y2 and Y3, were: 7.59% and 10.63% significantly ($P<0.05$) thicker than controls.

The average egg yolk cholesterol level in Group Y0 chickens was 7.75 mg/g of yellow yolk (Table 3) and showed no significant difference ($P>0.05$) compared to Group Y1 chickens. The average cholesterol level in egg yolks in Group Y2 and Y3 chickens was: 25.81% and 23.61% significantly ($P<0.05$) lower than controls.

Supplementation from 0.20 to 0.30% YC in the ration, significantly increased yolk color. This is due to the presence of YC probiotics that can cause digestive processes in the digestive tract to be better, so that it will increase feed consumption, which has an impact on increasing the consumption of xantophyl substances in yellow corn. According to Bidura (2019), the color of the yolk is very large influenced by the carotin content in the ration. So, the higher the consumption of feed, the higher the absorbed carotin which will affect the color of the yolk. The results of this study are in accordance with the Carvalho *et al.* (2019) which reported that supplementation of probiotic *Saccharomyces spp.* in the ration significantly increased yolk color and egg production.

Eggshell thickness in chickens given 0.20-0.30% YC probiotics increased significantly. Probiotic YC can increase feed consumption and digestibility of nutrients including calcium minerals which causes eggshell thickness to increase. As reported by Zhen *et al.* (2019) that supplementation of culture yeast in feed at a level of 0.8% can significantly improve the digestibility of Calcium and Phosphorus. This is the same as the opinion of Bidura *et al.* (2016) and Bidura (2019) that probiotic supplementation of *Saccharomyces spp*. in the ration at the level of 0.20 to 0.60% can significantly increase eggshell thickness and total egg weight in laying hens.

Cholesterol levels in chicken egg yolks that received supplementation of 0.20-0.30% YC were lower than control chickens. Cholesterol content in egg yolks is influenced by the amount of cholesterol distributed from the digestive tract of chickens. The presence of yeast *Saccharomyces spp* is able to produce an enzyme called Bile Salt Hydrolase (BSH), an enzyme that can de-conjugate bile salts (Bidura *et al*., 2012). Furthermore, bile salts will be excreted through feces, so the amount of bile acids returning to the liver becomes reduced (Ramasamy *et al*., 2010). To balance the concentration of bile salts, the body will take blood cholesterol (LDL) as a precursor. This process will ultimately reduce overall cholesterol levels. Reported by Zurmiati *et al.* (2014) that probiotics in the digestive tract can inhibit the absorption of cholesterol so that cholesterol synthesis in the body decreases. Probiotics that can degrade cholesterol into coprostanol, which is a sterol that cannot be absorbed by the intestine, then together with the rest of the cholesterol is excreted with feces (Zhuang *et al.*, 2012). The results of this study are the same as the Bidura study (2019); Bidura *et al.* (2016), and Ristiani *et al.* (2017) reported that supplementation of 0.20 to 0.40% probiotic *Saccharomyces spp.* in the ration significantly reduced cholesterol content in meat and decreased deposition of body fat in chickens. The research results of Puspani *et al.* (2016) reported that supplementation of probiotic Cellulolytic B-7 bacteria at the level of 0.20-0.60% in feed, could significantly reduce serum cholesterol content and the amount of abdominal fat in ducks. In contrast, Abdel-Hafeez *et al*. (2017) reported that probiotics in the ration had no significant effect on protein content and total cholesterol in blood serum, but markedly reduced carcass fat content in chickens.

CONCLUSION

It was concluded that supplementation of 0.20-0.30% Yeast culture (*Saccharomyces spp*.) in the ration could significantly increase egg production, feed efficiency, eggshell thickness and egg yolk color in Lohman Brown laying hens aged 70-80 weeks. Conversely, significantly reduce cholesterol content in egg yolks.

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Conflict of Interest Declaration

We declare that there are no conflicts of interest with financial organizations regarding this text.

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