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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

I Gusti Nyoman Gde Bidura[1*], Ida Bagus Gaga Partama[1], Dewa Ketut Harya Putra[1] and Urip Santoso[2]

[1]Faculty of Animal Husbandry, Udayana University, Denpasar-Bali, Indonesia
[2]Department of Animal Science, Faculty of Agriculture, Bengkulu University, Indonesia
**Corresponding author*

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A B S T R A C T

The current study was aimed to study the effect of addition on diet of various levels of probiotic *Saccharomyces spp.SB-6*, isolated from colon of Bali cattle, on egg productions and its total cholesterol levels of Lohmann Brown layers. A total number of 120 layers at 32 weeks of age and with homogenous body weight were randomly divided into 4 treatment groups. Each treatment group consisted of 6 replications and with 5 birds in each of it. Therefore, 24 battery colony pens were provided. The four treatment groups were assigned based on their diets, namely treatments B, C, and D in which their diets were supplemented with 0.20, 0.40, and 0.60 g of probiotic/kg of diet, respectively, whereas there was no probiotic supplementation for treatment A which was considered as the control group. All experimental birds were fed *ad libitum*. Six eggs from each treatment group were collected randomly for assessment of cholesterol content following standard procedure. The present results showed that, compared to control birds, layers that subjected to probiotic supplementation have a significant increase ($P<0.05$) in egg production (hen-day production) and in the total egg weight, but showed a slight decrease in serum and egg cholesterol contents. Thus, it may be concluded that addition on diet of probiotic *Saccharomyces spp.SB-6* at various level has positive effects on chicken production and, therefore, it can be recommended widely to chicken farmers.

Introduction

It is widely recognized that, in most developing countries, people need to increase their animal protein consumption. Thus, attempt must be made to enhance livestock production, particularly those that have rapid growth rate and short generation time such as poultry.

Concerning poultry production, while efforts have been made to improve the production, the low level of people's consumption of animal protein is further constrained by the ignorance and unwilling of people to eat eggs due to its high cholesterol content that is believed may lead to health problem.

In regard to improvement in poultry production, the use of antibiotics as growth promoter has been banned in Europe and the United States (Ahmad, 2006). Some other substances used to stimulate faster growth rate have also been banned, such as hormones; for example, the use or implantation of Stilboestrol in chicken has long been prohibited. Such prohibition also applies in Indonesia. Therefore, other ways or other approaches to enhance poultry production should be discovered or at least become topic of research work by related scientists.

It has been reported recently that the application of probiotic has led to many advantages for poultry farming. The initial findings showed that newly-hatched chicken can be protected from colonization by *Salmonella enteridis* when suspension of gut content obtained from healthy adult chickens was added on their diet (Ahmad, 2006). Probiotic can be considered as food ingredient consisting of live microbes that have beneficiary effect on health status. They may live and adhere to specific areas of the gastrointestinal tract and compete with the existing pathogens or harmful antigens (Dinkci *et al*., 2006). The addition of probiotic on diet are expected to increase the role of normal flora in the digestive tract of chicken which, in turn, may increase production of exogenous enzymes such as amylase, protease, and lipase, following which it may lead to an increase in activities of endogenous enzymes in hydrolyzing feed nutrients (Putra *et al*., 2015).

Generally, probiotics are derived from bacteria, fungus, and yeast. *Saccharomyces cerevisiae* is one of the well-known yeasts that has widely put in trade and, thus, given to livestock. Bidura (2007) has noted that supplementation of yeasts on diet may improve the quality of diet and consequently livestock performances; feed efficiency is also improved (Bidura *et al*., 2012). Furthermore, the beneficial effect of supplementation of probiotics has also been reported by some research workers such as Santin *et al*. (2001), Iji *et al*. (2001), Ghasemi *et al*. (2006), Mohiti *et al*. (2007), Yousefi and Karkoodi (2007), Suryani and Bidura (2000), Yamada and Sgarbieri (2005), Queiroz *et al*. (2004) and Roni *et al*. (2014). On the other hand, some authors (such as Ayanwale *et al*., 2006) reported that yeasts supplementation on diet has no effect on performances.

In addition to its effect on improved egg production, supplementation of probiotic on chicken diet may reduce egg cholesterol level and this may encourage people to consume eggs more without worrying about health problems that may occur. Referring to the initial finding mentioned above and by considering that source of probiotic is easily available locally, the work reported in this paper was conducted in order to study the effect of supplementation on diet of probiotic, obtained from colon of Bali cattle slaughtered at the local abattoir, on egg production and its cholesterol concentration in Lohmann Brown laying hen.

Materials and Methods

A total number of 120 Lohmann Brown layers at 32 weeks of age and with homogenous body weight were randomly divided and caged in 24 separate pens with 5 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 56 days (8 weeks). Eggs were collected three times a day and they were all weighed and recorded. For the purpose of examination of their cholesterol concentration, six eggs 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 (Scott *et al*., 1982). Various levels of *Saccharomyces spp*., obtained from colon of Bali cattle 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 5 birds in each replicate; therefore, the total number of experimental animals used in the current study was 4 x 6 x 5 = 120 birds. They were all provided with feed and drinking water *ad libitum* and vaccinated against Newcastle Disease and infectious bronchitis.

The probiotic used in the current study was *Saccharomycesspp.* SB-6, which was isolated from colon of Bali cattle 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*., 2014a). 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 total egg weight. Moreover, the supplementation of 0.20 – 0.60% of *Saccharomyces spp*. culture significantly ($P < 0.05$) increased the number of eggs produced (eggs/56 days). However, no significant different ($P>0.05$) in feed efficiency was noted. Similarly, the average feed intake of the experimental birds during the course of experiment did not differ significantly ($P>0.05$) among the treatments (Table 1).

Furthermore, the present results also found that supplementation of the probiotic at levels 0.20 – 0.60% has resulted in a significant ($P<0.05$) decrease in levels of cholesterol in the plasma and eggs. In regard to the percentage of hen-day production, it was recorded that the highest value was for layers subjected to treatment C – 0.40% probiotic supplementation.

The non-significant different in feed intake and feed conversion ratio – ratio between the total feed intake and the total egg weight – following probiotic supplementation noted in the current study is in accordance with previous study reported by some research workers for laying hens (e.g. Wibawa *et al*., 2014; Mohiti *et al*., 2007; Ayanwale *et al*., 2006; Yousefi and Karkoodi, 2007) and for broilers (Chumpawadee *et al*., 2008; Karaoglu and Durdag, 2005). They studied the effect of yeasts inclusion in the diet and noted that feed intake was not affected by the supplementation.

Concerning feed conversion ratio, the present results found that there was no significant different among the experimental treatments on feed efficiency. Similar conclusion was reported by Wibawa *et al*. (2014) who found that addition of 0.20% of *Saccharomyces spp*. culture on diet had no

effect on feed intake and feed efficiency of layers and broilers.Other previous workers also reported such a non-significant effect of inclusion on the diet of probiotic on feed efficiency (Mutus *et al*., 2006; Wahyuni *et al*., 2008; Suryani and Bidura, 2000; Yousefi and Karkoodi, 2007; Chumpawadee *et al*., 2009). On the other hand, some studies showed that probiotic supplementation on feed led to improvement in feed conversion ratio of broilers as reported by Puspani *et al*. (2014) and Umiarti *et al*. (2014). As stated by Mahdevi *et al*. (2005), the reasons for such varies effect of probiotic supplementation may be related to variations in gut flora and in environmental conditions. Thus, the inconsistent results about the effect of probiotic on poultry production may be due to several aspects such as strains of bacteria, dose of supplementation, diet composition, feeding strategy, form of feed and interaction with other dietary feed additives (Chesson, 1994).

The beneficial effects of supplementation of yeast product on poultry production have been reported by some research workers. It has been demonstrated that it may increase nutrient digestibility (Bidura *et al*., 2009; Bidura *et al*., 2012), reduce ammonia in excreta and increase microorganism population in the gastro-intestinal tract (Jin *et al*., 1997). The mechanism of action of yeast product, however, has not been completely understood. Yeast culture may provide various growth factors, pro-vitamin and other stimulants for bacterial growth in the gastro-intestinal tract (Ahmad, 2006). *Saccharomyces cerevisiae*, in particular, has been used in animal feed for several decades and is considered as a source of high quality protein, B-complex vitamin, selenium and zinc (Queiroz *et al*., 2004).

Although yeast supplementation in the diet did not affect the feed intake and feed efficiency, it may significantly increase the total egg weight and egg production (hen-day production) as reported by Bidura *et al*. (2014b), Wahyuni *et al*. (2008) and Warmadewi *et al*. (2009). Moreover, Piao *et al*. (1999) noted that the use of 0.10% yeast (*Saccharomyces serevisiae*) 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% *Saccharomycesspp* culture significantly improved live weight gain 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).

The present results demonstrated that *Saccharomyces* spp supplementation increased egg production and its total weight. This may be due to the fact that the present of probiotic in the digestive tract of poultry may improve digestibility of nutrients, particularly of protein and minerals (Bidura *et al*., 2012). It has been reported earlier (Yi *et al*., 1996) that supplementation of microbe in the diet improved nitrogen retention in broiler and *in vitro* digestibility of vegetable protein.

Other studies (Chen *et al*., 2005; Yi *et al*., 1996; Hong *et al*., 2004) also recorded that addition of 0.20% complex probiotics consisting of *Lactobacillus acidophilus* and *Saccharomyces serevisiae* on basal diet increased digestibility of dry matter and crude protein.

Piao *et al*. (1999) suggested that probiotic in the gastro-intestinal tract may improve

protein and energy retention in the body of birds. According to Bedford and Classen (1992), fungal probiotics are effective in degrading complex compound such as beta-glucans and arabinoxylans and supplementation of microbe in diet may improve the dietary bioavailability (Wang *et al*., 2004; Chen *et al*., 2005; Cho *et al*., 2007). Further results showed that *Saccharomycesspp*. is yeast that capable of producing enzymes such as amylase and cellulase (Utama, 2011) so that they may increase digestibility of protein and crude fibers such as cellulose and hemi-cellulose to form simple mono-saccharides. Moreover, as stated by Howard *et al*. (2003), cellulolytic yeasts are capable of producing enzymes 1,4 beta-endo-glukonase, 1,4 beta-exo-glukonase and beta-glukosidase that may degrade components of crude fibers into soluble carbohydrates.

Table.1 Feed Efficiency, Hen-Day Production, and Egg Cholesterol Levels of Layers Following Supplementation on Diet of Various Levels of Probiotic *Saccharomyces Spp*.Sb-6

Variables	Supplementation of probiotic *Saccharomyces spp.*SB-6 in the ration (%)				SEM[1)]
	0.00	0.20	0.40	0.60	
Feed consumption (g/d)	159.90a[2)]	160.29a	161.18a	160.87a	1.072
Total egg weight (g)	2774.17b	2954.72a	2959.72a	2920.76a	20.705
The number of eggs (eggs/56 days)	45,93b	48,03a	48,25a	47,96a	0,826
Feed conversion ratio[3)]	3,23a	3,04a	3,05a	3,08a	0.037
Hen-day production (%)	82.02b	85.77a	86.64a	85.64a	1,035
Serum cholesterol (mg/dl)	186.38a	167.03b	159.92b	165.37b	4.947
Egg cholesterol (mg/dl)	578.29a	524.82b	509.16b	516.46b	15.173

Note:

1. *Standart error of the treatment means*
2. *Means with different superscripts within rows are significantly different (P<0.05)*

Comparison between feed intake with total egg weight

The present results showing that supplementation of culture of *Saccharomyces spp. SB-6* isolated from colon content of Bali cattle that may lowered cholesterol levels in serum and eggs is in accordance with results reported by Onifade *et al*. (1999). They found that addition of innocuous microorganisms including yeasts on diet of rabbits and broiler chickens resulted in a decrease in serum cholesterol, triglycerides and phospholipids. 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; Suryani and Bidura, 2000). 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.

Fungi have been recognized as microbe that may produces high concentration of lipase (Ulker *et al*., 2010). It may hydrolyze the lipid content of diet. Sutarpa *et al*. (2011) stated that the use of probiotic on diet may significantly lowered levels of cholesterol in serum and in meat of native local chickens. Probiotic (*S. cereviseae*) at an appropriate level of supplementation may reduce serum and egg cholesterol concentrations and improve hen-day egg production. Therefore, its inclusion on diet at level of 1.0 g/kg ration of layers can be recommended for optimum hen-day egg production and for lowering the concentrations of cholesterol in serum as well as in eggs (Ezema and Eze, 2015).

As a conclusion, the implementation on diet of probiotic *Saccharomyces* spp.SB-6, isolated from colon content of Bali cattle and at appropriate levels of inclusion, may significantly reduce serum and egg cholesterol concentrations and improve hen-day egg production. Therefore, based on the present results, its inclusion at level of 4.0 g/kg of layer diets can be recommended in order to obtain optimum hen-day egg production and minimum serum and egg cholesterol concentrations.

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