

**Implementation on Diet of Probiotic *Saccharomyces spp.*  
*Gb-7* and *Gb-9* Isolated from Colon of Native Chickens on  
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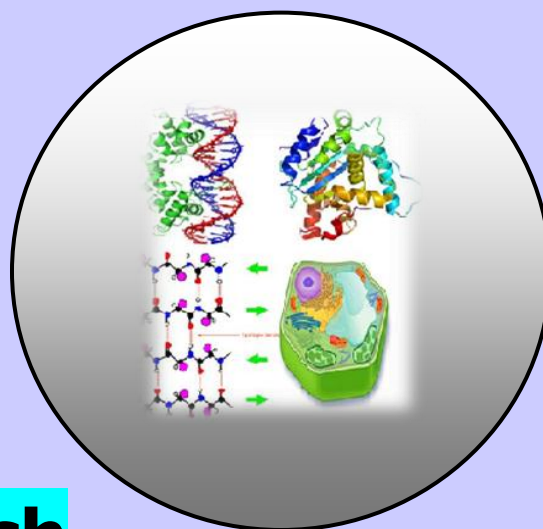
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**Implementation on Diet of Probiotic *Saccharomyces spp.* Gb-7 and Gb-9 Isolated from Colon of Native Chickens on Performance and Cholesterol Serum of Broiler**  
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**ABSTRACT**

The current research was conducted in order to evaluate the effect of two isolates of probiotics (*Saccharomyces spp.* Gb-7 and Gb-9) isolation from colon of native chickens on total serum cholesterol and performance in broilers. A total 240 broilers were randomly divided into 4 groups (A,B,C, and D) of 10 birds each. The diets for group A, B, and C were supplemented with probiotics 0.30% *Saccharomyces spp.* Gb-7, 0.30% *Saccharomyces spp.* Gb-9, and 0.15% *Saccharomyces spp.* Gb-7+0.15% *Saccharomyces spp.* Gb-9 of feed, respectively. Group D diet had no probiotic (control). 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 commercial kit. The result showed that the probiotic fed groups had a significant ( $P<0.05$ ) reduction in serum cholesterol levels than the control (group D). The bird consumed probiotic *Saccharomyces spp.* had higher live weight gain (LWG)s and were efficient in using feed compared to those unsupplemented probiotics in their diets ( $P<0.05$ ). It was concluded that using of probiotics (*Saccharomyces spp.* Gb-7; Gb-9; and its combinations) isolation from colon of native chickens could increase growth performance and feed efficiency of broiler up to six weeks of age. Moreover it decreased the serum cholesterol contents of birds.

**Key words:** *Saccharomyces spp.*, Probiotics, Performance and Cholesterol.

**INTRODUCTION**

Probiotics are live microbial food ingredients which are beneficial to health. This probiotic action includes survival in and adhesion to specific areas of the gastrointestinal tract and

competitive exclusion of pathogens or harmful antigens (Dinkçi *et al.*, 2006). Currently, most probiotics have been selected from members of the normal healthy adult microbiota. The strains with beneficial properties, which are potential sources of probiotics most frequently belong to the general are *Bifidobacterium* and *Lactobacillus* (Isolauri, 2004). Use of antibiotics as an additive in poultry diets to improve growth has been banned in several country. A popular alternative to the use of antibiotics has been use of probiotics (Chumpawadee *et al.*, 2009).

The use of *Saccharomyces spp* culture as probiotic source in poultry production becomes an area of great interest. Gut microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine. Previous experiments showed that the inclusion of microorganisms in the diets improved feed conversion efficiency and digestibility (Chen *et al.*, 2005). 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). 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). Dinkci *et al.* (2006) reported tha 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.

Generally, probiotics are derived bacteria, fungi and yeast. *Saccharomyces cerevisiae*, one of the most widely commercialized types of yeast, has long been fed to animals. Live yeast addition to animal feed has been known to improve the nutritive quality of feed (Candrawati *et al.*, 2014; Bidura *et al.*, 2014; Bidura *et al.*, 2015) and performance of animals (Bidura *et al.*, 2012; Puspani *et al.*, 2014). In addition, mannan oligosaccharides and fructo oligosaccharide derived from the cell wall of the yeast *S. cerevisiae*, has shown promise in suppressing enteric pathogens, and modulating the immune in studies with poultry (Santin *et al.*, 2001; Spring *et al.*, 2000; Iji *et al.*, 2001).

Additionally, there are trials showing that enrichment of diets with yeast could favorably improve the feed efficiency (Bidura *et al.*, 2012), growth rate (Bidura *et al.*, 2009; Puspani *et al.*, 2014; Ghasemi *et al.*, 2006), egg productions (Mohiti *et al.*, 2007), and egg quality (Yousefi and Karkoodi, 2007). Putra *et al.* (2015) reported that the addition of probiotics in diet are expected to increase the role of the normal flora in the digestive tract of poultry to produce exogenous enzymes such as amylase, protease, and lipase which can increase the activity of endogenous enzymes to hydrolyze the feed nutrients.

The addition of probiotic to feed aimed to increase the population of probiotic in tilapia digestive tract so that the action mechanism of the probiotic in producing exogenous enzymes for digestion probiotic in producing exogenous enzymes for digestion will increase (Merrifield, 2010; Cerezuela *et al.*, 2011).

Furthermore, the beneficial effect of supplementation of probiotics has also been reported by some research workers such as Ghasemi *et al.* (2006), Mohiti *et al.* (2007), Yousefi and Karkoodi (2007), Yamada and Sgarbieri (2005), and Queiroz *et al.* (2004). Supplementation of 0,20-0,30% *Saccharomyces spp.* culture in basal diets were increased body weight, but decreasing abdominal-fat and excrete ammonia-N concentration of bali drake up to eight weeks of age (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.

The objective of this study was in order to evaluate the effect of two isolates of probiotics (*Saccharomyces spp.* Gb-7 and Gb-9) isolation from colon of native chickens on total serum cholesterol and performance in broilers.

## MATERIAL AND METHODS

**Animals, treatments, and experimental design:** A total of two hundred and forty 2-wk-old broiler, were obtained from a local commercial broiler farm with an average body weight of ( $504.82 \pm 10.61$  gram). The all chicks were fed of comercial feed broiler containing ME, 3,200 kcal/kg; CP, 22%; Ca, 2.0%; available phosphorus, 0.65% from 15-42 days of age. Feed and water were provided *ad libitum*. A total 240 broilers were randomly divided into 4 groups (A, B, C, and D) of 10 birds each. The diets for group A, B, and C were supplemented with probiotics 0.30% *Saccharomyces spp.* Gb-7; 0.30% *Saccharomyces spp.* Gb-9; and 0.15% *Saccharomyces spp.* Gb-7+0.15% *Saccharomyces spp.* Gb-9 of feed, respectively. Group D diet had no probiotic (control). Broiler chickens were weighed individually on a weekly basis and both feed consumption was recorded daily.

**Khamir strains and khamir identification:** The probiotic used in the current study were isolates *Saccharomyces spp.* Gb-7 and Gb-9, which was isolated from colon of native chickens slaughtered at the local abattoir. The isolate has passed the test and qualified as potential probiotic according to our previous study (Bidura *et al.*, 2016).

**Sampling and laboratory analysis:** At the end of experiment (42 days of age), 18 broiler chickens in each treatment groups were selected and slaughtered. In order to avoid variations in the cutting procedures, the same operator was employed (Zhang *et al.*, 2013). For analysis of total serum cholesterol, two ml of blood was taken from the jugular vein of each birds 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 commercial kit.

**Statistical analysis:** Performances of broiler and cholesterol contents in their serum were analyzed using one way analysis of variance and if it was significantly different it was further tested by Duncan's Multiple Range Test.

## RESULTS

Results of the current study are presented in Table 1. It can be noted that supplementation on diet of two isolates of probiotics (*Saccharomyces spp.* Gb-7; Gb-9; and it's combination)

isolation from colon of native chickens resulted in a significant ( $P<0.05$ ) increase in live weight gains and feed efficiency (Table 1). However, the average feed consumption of the experimental birds during the course of experiment did not differ significantly ( $P>0.05$ ) among the treatments (Table 1).

The average value of feed conversion ratio (feed consumption : weight gain) during the four weeks of observation birds were given the control treatment was 1.87/head (Table 1). The average of the value of FCR in birds treatment A, B, and C, were 13.90%; 11.76%; and 12.83% ( $P<0.05$ ) lower than the control (D), respectively.

Furthermore, the present results also found that supplementation of two isolates of probiotics (*Saccharomyces spp.* Gb-7; Gb-9; and it's combination) isolation from colon of native chickens in diets has resulted in a significant ( $P<0.05$ ) decrease in levels of cholesterol in the plasma.

**Table 1. The effect of two isolates of probiotics (*Saccharomyces spp.* Gb-7 and Gb-9) isolation from colon of native chickens on total serum cholesterol and performance in broilers (2-6 weeks of age).**

Variables	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
	Group A	Group B	Group C	Group D	
Live weight gains (g/head/4 weeks)	1804.27 a <sup>1)</sup>	1839.05 a	1815.81 a	1606.39 b	52.925
Feed Consumption (g/head/4 weeks)	2904.87 a	3034.43 a	2959.77 a	3022.65 a	65.705
FCR (feed consumption: weight gain)	1.61 b	1.65 b	1.63 b	1.87 a	0.026
Serum cholesterol (mg/dl)	142.71 b	138.39 b	143.52 b	169.93 a	7.283

Notes:

1. The diets for group A, B, and C were supplemented with probiotics 0.30% *Saccharomyces spp.* Gb-7; 0.30% *Saccharomyces spp.* Gb-9; and 0.15% *Saccharomyces spp.* Gb-7+0.15% *Saccharomyces spp.* Gb-9 of feed, respectively.
2. SEM : "Standard Error of Treatment Means"
3. Means with different superscripts within rows are significantly different ( $P<0.05$ )

## DISCUSSION

The data indicated that there were no significant difference ( $P>0.05$ ) in feed consumption. The results are in agreement with previous studies in laying hens (Mohiti *et al.*, 2007; Ayanwale *et al.*, 2006; Yousefi and Karkoodi, 2007) and broilers (Chumpawadee *et al.*, 2008; Karaoglu and Durdag, 2005) that observed feed intake was not affected by yeast inclusion in the diet. They studied the effect of yeasts inclusion in the diet and noted that feed intake was not affected by the supplementation.

Additionally, feed conversion ratio was significant different among treatment. These results are in unagreement with those of Mutus *et al.* (2006) who reported that the supplement of a probiotic did not have any effect on feed conversion ratio. Yousefi and Karkoodi (2007) also reported that feed conversion ratio was not affected by the dietary probiotic and yeast supplementation. In addition Chumpawadee *et al.* (2008) could not detect any difference in the feed conversion ratio of the broilers as compared to the control. On the other hand, some studies show that probiotics supplementation in the feed of chickens improve the feed conversion ratio (Umiarti *et al.*, 2014; Bidura *et al.*, 2009; Bidura *et al.*, 2012; Bidura *et al.*, 2014). The reason for the variable effect of biological additives may be confounded by variations in gut flora and environmental condition (Mahdavi *et al.*, 2005). The viability of probiotic microbes depends on many factors, e.g., strain, medium composition and storage technology (Anadon *et al.*, 2006).

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 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). Santin *et al.* (2001) found that supplementation of feed with *S.cerevisiae* cell wall (0.2%) improved broiler body weight.

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 (Puspani *et al.*, 2014) 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).

Piao *et al.* (1999) suggested that probiotic in the gastro-intestinal tract may improve protein and energy retention in the body of birds. 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; Bidura *et al.*, 2009; Bidura *et al.*, 2015; Candrawati *et al.*, 2014). Further results showed that *Saccharomyces spp.* is yeast that capable of producing enzymes such as amylase and cellulase (Utama, 2011; Bidura *et al.*, 2015) 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.

These results are in unagreement with Ayanwale *et al.* (2006) who found that yeast (*S. cerevisiae*) supplementation in the diets of pullets did not have any effect of body weight gain. Chumpawadee *et al.* (2008) observed supplementation of cassava yeast to broiler diets did not improve growth rate. 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). 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 present results also found that supplementation of two isolates of probiotics (*Saccharomyces spp.* Gb-7; Gb-9; and it's combination) isolation from colon of native chickens in diets has decreased in levels of cholesterol in the plasma. 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).

De Smet *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 concentrattion. 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. cerevisiae*) 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).

There are controversial results regarding the effect of probiotic on lipid profile. The mechanisms involved may be as follows (Rao *et al.*, 2006): (i) the bacteria will bind with cholesterol which result in inhibiting the absorption of cholesterol back into the body; (ii) cholesterol elimination in feces will be facilitated by bacteria; (iii) cholesterol synthesis enzymes will be inhibited via fermentation products of lactic acid bacteria therefore, cholesterol production will be decreased; (iv) interference of the bacteria in the recycling of bile salt (a metabolic product of cholesterol) and facilitate its elimination which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption; and (v) the assimilation of lactic acid.

As a conclusion, the implementation on diet of probiotic *Saccharomyces* spp.Gb-7; Gb-9; and it's combination isolates (isolated from colon of native chickens) and at appropriate levels of inclusion, may significantly increased performance and reduce serum cholesterol concentrations of broiler up to six weeks of age.

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