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Research Article
Effect of Probiotic *Saccharomyces* spp. on Duck Egg Quality Characteristics and Mineral and Cholesterol Concentrations in Eggshells and Yolks

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Abstract

Objective: The present study was conducted to determine the dosage effects of *Saccharomyces* spp. Kb-5 (isolated from buffalo rumen fluid) as a probiotic on egg production performance, egg quality characteristics and mineral and cholesterol concentrations in the yolks of eggs from laying ducks. **Materials and Methods:** Two hundred and forty 40-week-old healthy laying ducks (*Anas* sp.) were used in a completely randomized design divided into four treatment groups: S0, without administration of the probiotic; S1, with administration of 0.10% *Saccharomyces* spp. Kb-5; S2, with administration of 0.20% *Saccharomyces* spp. Kb-5 and S3, with administration of 0.30% *Saccharomyces* spp. Kb-5. **Results:** This study showed that administration of *Saccharomyces* spp. Kb-5 increased egg mass, feed efficiency, feed digestibility, yolk color, yolk and eggshell weight, shell thickness and Ca content in the eggshell and yolk ($p<0.05$) but did not increase the efficiency of feed consumption ($p>0.05$). Administration of 0.20-0.30% *Saccharomyces* spp. resulted in lower ($p<0.05$) yolk cholesterol contents. **Conclusion:** It is concluded that supplementation of laying duck diets with 0.20-0.30% *Saccharomyces* spp. Kb-5 increased egg mass, feed efficiency, feed digestibility, yolk color, yolk and eggshell weight, shell thickness and Ca contents in the eggshell but decreased yolk cholesterol contents.

Key words: Calcium, shell thickness, cholesterol, laying ducks, egg quality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In general, eggs are recognized as a complete source of protein and other valuable nutrients but eggs also contain high levels of cholesterol, which, if consumed in excess, can cause cardiovascular disease in humans. Therefore, the approach of reducing cholesterol content in eggs not only helps improve public health efforts but also can benefit the laying hen industry. Some recent studies have used dietary supplements from probiotic strains[1-6] to regulate serum and meat cholesterol concentrations, as well as to improve egg yolks and egg quality.

Antibiotics have been added to poultry diets for a long time. Continuous use of antibiotics can contribute to increased resistance to pathogenic bacteria and is predominant in animal products, which can have a negative impact on the health of the humans that consume them.[7,8] As a result, increasing concerns about antibiotic resistance led to a ban on antibiotic growth promoters (AGPs) in the European Union in 2006; thus, alternatives to AGPs are needed[9,10].

Probiotics have proven to be the alternative most preferred by breeders and are effective as a substitute for AGPs for inhibiting pathogenic bacteria in the poultry industry. At present, yeast *Saccharomyces* sp. is mainly used as a probiotic in the diet of ducks, broilers and laying hens[2,3,11-14] to improve the nutritional quality of feed[15-18] and to reduce abdominal fat and serum cholesterol levels in ducks and broilers[11-20] and egg cholesterol levels in laying hens[11]. There are two possible mechanisms for the beneficial effects of probiotic bacteria on digestive disorders: (1) The production of antimicrobial compounds, such as lactic acid and bacteriocin and (2) Adherence to the mucosa and coaggregation to form a barrier that prevents colonization by pathogens[21]. The optimal level of addition of probiotics to diets to improve growth performance, nutrient digestibility and a decrease in harmful gas emissions in pigs was obtained when probiotic mixtures were added at 0.30%[22], the excretion of N and P in feces decreases with supplementation of probiotics in feed[23]. Probiotics in the digestive tract can increase food digestibility and protein retention as well as the concentrations of the minerals Ca, Co, P and Mn[24,25], Increased retention of Ca and P increases eggshell thickness.

Previous studies have shown that *Saccharomyces* spp. isolates from buffalo rumen have potential as probiotics and have the ability to degrade crude fiber[15]. *Saccharomyces cerevisiae* has the potential to assimilate cholesterol in vitro[26]. The application of *Saccharomyces* spp. Kb-5 in broiler diets improved growth performance and feed efficiency but reduced serum and meat cholesterol levels in male ducks[2] *Saccharomyces* spp. Kb-5 can increase the crude protein and metabolizable energy (ME) of rice bran[15]. Probiotic supplementation does not improve the performance of laying hens. However, beneficial effects on the quality of eggshells result from probiotic supplementation[27].

Probiotic supplementation in duck drinking water significantly increased income compared to the cost of feed and increased the total small intestine *Bacillus* sp. and the number of bacterial colonies in ducks[28]. The digestibility coefficient of dry matter, organic matter, crude protein (CP), neutral detergent fiber and gross energy is higher in groups of animals given probiotics than in the control group or in groups not given probiotics[29].

Multispecies probiotic preparations are considered to be more effective than single probiotic strains. Probiotic yeast such as *Saccharomyces* sp. has also been shown to stimulate the immune system of chicks without reducing growth performance[30]. Therefore, this study aimed to evaluate the effect of the probiotic inclusion level of *Saccharomyces* spp. (isolated from buffalo rumen fluid) on production performance, egg quality characteristics, egg yolk cholesterol and the fatty acid profile of egg yolks of laying ducks.

MATERIALS AND METHODS

Animal treatments and experimental design: Two hundred and forty 40-week-old healthy laying ducks (*Anas* sp.) were used in a completely randomized design experiment. All diets were isonitrogenous (CP: 16%) and isoenergetic (ME: 2900 kcal kg^{-1}). The diets were formulated to meet the nutrient requirements for poultry[31] for the 16 weeks of the experiment. Laying ducks were randomly divided into four treatment groups, namely, S0: without administration of probiotics, S1: feed with the addition of 0.10% *Saccharomyces* spp. Kb-5, S2: feed with the addition of 0.20% *Saccharomyces* spp. Kb-5 and S3: feed with the addition of 0.30% *Saccharomyces* spp. Kb-5. Each treatment consisted of six replication cages of 150×70×45 cm (length×width×height) with 10 birds randomly assigned to each cage. Each experimental diet was in the form of mash and birds had free access to feed and water during the experiment. The probiotic used in this study was *Saccharomyces* spp. Kb-5, which was isolated from fluids from the rumen of buffalo slaughtered at the local abattoir. The isolates had passed the test and were considered a potential probiotic according to our previous research[15]. The *Saccharomyces* spp. Kb-5 isolate was prepared as a probiotic at the Laboratory of Nutrition and Technology at the Faculty of Animal Husbandry, Udayana University. Every 1 g of

Table 1: The ingredients and calculated nutrient content of the feed for laying ducks up to forty weeks old

Basal diet	Composition
Ingredients (%)	
Yellow corn	60.20
Rice bran	17.00
Soybean	9.20
Fish meal	9.17
Mineral-B12 *)	4.43
Total	100.00
Metabolizable energy	2900 (kcal kg^{-1})
Chemical composition**	
Crude protein	16.0%
Crude fiber	3.90%
Ether extract	7.12%
Calcium	2.93%
Phosphorus	1.1%
Arginine	1.16%
Lysine	1.07%
Methionine+cysteine	0.69%
Tryptophan	0.20%

*The mineral composition of B12 per 10 kg contains: Calcium: 49%, Phosphorus: 14%, Iron: 40000 mg, Manganese: 27500 mg, Mg: 27.500 mg, Zinc: 25 mg, Vit B12: 4.50 mg and Vit D3: 500000 IU. PT. Eka Farma Deptan RI No. D 8109127 FTS. **Based on calculations in Scott *et al.*[32]

culture contained at least 4.9×10^7 CFU *Saccharomyces* spp. The ingredients and chemical compositions of the feed are shown in Table 1.

Live performance: Continuous lighting and access to feeding and water were provided during the experiment. The birds were weighed at the start (age 40 weeks) and the end (age 56 weeks) of the experiment. Eggs were collected every day and egg production is expressed daily (%hens day^{-1}). Individual egg weights were recorded and then used to calculate the average egg weight for all trial periods. The total egg mass was calculated by multiplying the weight of the egg by egg production. Feed intake was measured by cage (10 ducks) every week. Daily feed intake per bird was calculated based on the total cage intake for the entire trial period and for the number of days in all periods. The feed conversion ratio (kg of feed kg^{-1} of eggs) for all periods was calculated based on cage egg production, egg weight and feed consumption. Egg quality parameters were measured using a multi egg tester.

Quality of eggs and yolk minerals: Examination of the eggs and eggshell quality (shell weight, eggshell thickness, egg yolk and albumin weight and egg yolk color) was carried out at the end of the experiment. For this purpose, four eggs per cage were taken randomly from each group between 08.00 and 12.00 h at 56 weeks (a total of 12 eggs per group during the experiment). Eggs were weighed individually and the specific gravity of the eggs, egg index and shell thickness were measured. After the egg was broken on the EQM measurement stand, the albumen and yolk height were measured. The intensity of the yolk color was evaluated and recorded according to the Roche Yolk Color Fan method. The albumen weight was calculated by subtracting the weight of the yolk and shells from the overall egg weight. To measure the weight of the shell, the eggshell was cleaned of the albumen that attached to the membrane; eggshells were then dried at room temperature and their weight was expressed as a percentage of the total egg weight. Evaluation of egg quality was carried out on individual eggs, similar to the method for evaluating the weight of the eggs. The mineral concentration of the egg yolk (Ca and Mg) was measured by the AAS method. Egg yolk samples were analyzed for dry matter content (DM) by drying the sample at 105°C for 24 h in a forced air oven. The total yolk cholesterol content was analyzed following the Liebermann-Burchard method[33].

Retention and excretion of nutrients: Nutrient digestibility values (dry matter and organic matter digestibility) were determined. The amount of feed used was 120 g and this amount was based on a preliminary test with consumption of laying duck rations. All birds were fasted for 24 hours to ensure that their digestive tract was empty of leftover feed. They were then force-fed the assigned diets (all treatments). Stainless steel funnels with 40 cm stems were used in the forced feeding techniques[2]. Water was available *ad libitum* during the trial period. Total excreta was collected in plastic trays. The stool samples were frozen, allowed to reach equilibrium with atmospheric humidity, weighed and pounded through a 1 mm filter. Excreta samples and diets were analyzed to determine dry matter (DM) and organic matter (OM), respectively. Dry matter (DM), organic matter (OM) and ash determination were carried out in accordance with the methods of the Official Analytical Chemistry Association[34]. All tests were carried out in triplicate.

Statistical analysis: All data were analyzed by one-way ANOVA to determine the differences among treatments. If differences were found, further analysis was carried out with Duncan's multiple range test ($p<0.05$).

RESULTS

The final body weight, feed consumption, egg production, dry matter and organic matter digestibility in the groups fed the experimental diets are shown in Table 2. The treated ducks exhibited significantly higher ($p<0.05$) egg

Table 2: Effects of probiotic *Saccharomyces* spp. Kb-5 (isolated from buffalo rumen fluid) level in diets on egg production and feed digestibility in laying ducks

	Groups				
Variables	S0	S1	S2	S3	SEM
Initial body weight (g)	1673.91[a]	1678.05[a]	1675.32[a]	1679.04[a]	28.903
Final body weight (g)	1704.71[a]	1699.73[a]	1726.48[a]	1738.02[a]	31.183
Feed consumption (g bird^{-1} weeks^{-1})	892.84[a]	885.37[a]	869.94[a]	875.38[a]	6.907
Egg production (%)	56.42[a]	56.29[a]	55.36[a]	54.95[a]	0.981
Egg mass (g bird^{-1} weeks^{-1})	218.82[b]	219.64[b]	230.15[a]	232.04[a]	2.972
Feed conversion ratio (feed consumption: egg mass)	4.08[a]	4.03[a]	3.78[b]	3.77[b]	0.259
Dry matter digestibility (%)	77.35[b]	77.02[b]	80.63[a]	81.72[a]	0.705
Organic matter digestibility (%)	79.38[b]	79.92[b]	82.65[a]	83.27[a]	0.698

S0: Basal diet (control), S1: Basal diet+0.10% *Saccharomyces* spp. Kb-5; S2: Basal diet+0.20% *Saccharomyces* spp. Kb-5 and S3: Basal diet+0.30% *Saccharomyces* spp. Kb-5, respectively. SEM: Standard error of treatment means. Means with different superscripts within raw values are significantly different (p<0.05)

Table 3: Effect of probiotic *Saccharomyces* spp. Kb-5 (isolated from buffalo rumen fluid) level in diets on external egg quality characteristics of laying ducks

	Groups				
Variables	S0	S1	S2	S3	SEM
Eggshell weight (%)	11.080[b]	11.490[b]	12.780[a]	12.930[a]	0.307
Egg yolk weight (%)	28.710[b]	28.650[b]	30.360[a]	30.520[a]	0.475
Egg albumen (%)	61.210[a]	59.860[a]	56.860[b]	56.550[b]	1.109
Haugh unit (white height:egg weight)	75.920[a]	76.080[a]	76.720[a]	76.590[a]	0.904
Egg shape (egg width/egg length) x100%	75.710[a]	74.980[a]	75.630[a]	76.580[a]	0.735
Specific gravity (weight:volume)	1.039[a]	1.047[a]	1.043[a]	1.051[a]	0.061
Shell thickness (mm)	0.438[b]	0.455[b]	0.518[a]	0.541[a]	0.0148
Eggshell mineral contents (ppm)					
Magnesium (Mg)	0.497[a]	0.505[a]	0.581[a]	0.597[a]	0.183
Calcium (Ca)	32.085[b]	33.102[b]	37.629[a]	37.492[a]	1.175

S0: Basal diet (control), S1: Basal diet+0.10% *Saccharomyces* spp. Kb-5, S2: Basal diet+0.20% *Saccharomyces* spp. Kb-5 and S3: Basal diet+0.30% *Saccharomyces* spp. Kb-5, respectively. SEM: Standard error of treatment mean. Means with different superscripts within raw values are significantly different (p<0.05)

mass, feed conversion ratio (feed consumption: egg mass) and feed digestibility than those of the control birds. No significant differences (p>0.05) in the final body weight, egg production, or feed consumption were observed among the treated groups.

The egg mass for ducks in Groups S2 and S3 was significantly higher (p<0.05) than those of the control (S0) and S1 Group during the total experimental period. There were no significant differences in feed consumption during the experimental period. The feed conversion ratio (feed consumption:egg mass) and feed digestibility in the groups fed probiotics (groups S2 and S3) were higher than those of the control group.

The effects of dietary supplementation with probiotic *Saccharomyces* spp. Kb-5 on the external egg quality characteristics of laying ducks are presented in Table 3. There were no significant differences (p>0.05) found between the treatment groups with respect to Haugh unit, egg shape, specific gravity, or Mg content in the eggshell. However, eggshell, yolk, shell thickness and Ca content in the eggshell were significantly different (p<0.05) and increased with 0.20-0.30% *Saccharomyces* spp. Kb-5 supplementation. However, the albumen content decreased significantly (p<0.05) with supplementation. The mineral content of Ca in the eggshells of the S2 and S3 groups were 17.27 and 16.85%, respectively, which were significantly different (p<0.05) from that in the control.

In addition, the results show that an additional *Saccharomyces* spp. (group S1, S2 and S3) in diets resulted in a significant (p<0.05) increase in yolk color and in Mg and Ca contents in the egg yolks (Table 4). The average yolk color in the S2 and S3 groups was significantly (p<0.05) increased by 10 and 15%, respectively, compared to the yolk color in the S0 treatment. The mineral content of Mg in the egg yolks of the S2 and S3 groups were significantly higher (p<0.05) than that in the control (20.24 and 25.07%, respectively). The mineral contents of Ca in the yolks of group S2 was 15.18%, followed by group S3, at 13.54%, which were significantly (p<0.05) higher than that of group S0 (control).

Egg yolk cholesterol levels with the probiotics *Saccharomyces* spp. (groups S2 and S3) also were significantly (p<0.05) lower in the eggs from Groups S0 and S1 (Table 4). The cholesterol content in yolks from group S2 was 18.61%, followed by group S3 at 17.80%, which was significantly (p<0.05) lower than that in group S0 (control).

Table 4: Effect of feeding *Saccharomyces* spp. Kb-5 (isolated from buffalo rumen fluid) in diets on yolk color and egg yolk mineral and cholesterol contents in laying ducks

	Groups				
Variables	S0	S1	S2	S3	SEM
Yolk color (1-15)	7.5200[b]	7.390[b]	8.3400[a]	8.2500[a]	0,217
Egg yolk mineral contents (ppm)					
Magnesium (Mg)	0.1907[b]	0.1998[b]	0.2293[a]	0.2385[a]	0,009
Calcium (Ca)	14.7047[b]	15.0259[b]	16.9372	16.6951[a]	0.418
Egg yolk cholesterol (mg g^{-1})	7.3600[a]	7.1600[a]	5.9900[b]	6.0500[b]	0.316

S0: Basal diet (control), S1: Basal diet+0.10% *Saccharomyces* spp. Kb-5, S2: Basal diet+0.20% *Saccharomyces* spp. Kb-5 and S3: Basal diet+0.30% *Saccharomyces* spp. Kb-5, respectively. SEM: Standard error of treatment mean. Means with different superscripts within raw values are significantly different (p<0.05)

DISCUSSION

Probiotics have been proven to be the most preferred and effective alternative to AGPs and pathogenic bacterial inhibitors in the poultry industry. At present, *Lactobacillus*, *Bifidobacterium* and yeast are mainly used as probiotic preparations in broilers and laying hens[35,3]. Among these probiotic microbes, *Bacillus* members have been considered the most promising because of their survival through digestion, germination in the digestive tract and excretion through excreta[36].

Supplementation of 0.20-0.30% with the yeast *Saccharomyces* spp. in duck diets led to higher egg mass, feed conversion ratio (feed consumption:egg mass) and feed digestibility than those in the control birds. As reported by Chen *et al.*[37], microorganisms, including *Lactobacillus*, *Bacillus* and *Saccharomyces cerevisiae*, have been studied as potential feed additives because they produce extracellular enzymes including proteases, amylases, cellulases and lipases. These enzymes can increase the digestibility of protein, carbohydrates and lipids in broilers[38]. The same was reported by Phuoc and Jamikorn[29], who found that supplementation with *Lactobacillus acidophilus* alone or in combination with *B. subtilis* at half a dose could increase the number of beneficial intestinal bacteria populations, nutrient digestibility, cecal fermentation, feed efficiency and growth performance in rabbits. Pigs that are fed probiotics had increased feed conversion ratios[39]. The inclusion of probiotics in the diet significantly increased the number of goblet cells and the villus length[40]. Azzam *et al.*[41] reported that various factors, such as microbial colonization in the intestine, can affect the production, secretion and composition of mucin. The higher synthesis of the mucin gene after administration of probiotics can positively influence bacterial interactions in the intestinal digestive tract, the proliferation of intestinal mucosal cells and consequently, the efficient absorption of nutrients[40]. According to Nguyen *et al.*[22], the largest increase in nutrient digestibility, fecal bacterial enumeration and harmful gas emissions in weaned pigs was obtained when a probiotic mixture was added at 0.3%.

Better feed conversion was seen in the duck group that received probiotics compared to the control group, which proved to be the reason for the increase in egg weight, because almost all treatments had the same feed intake. The same result has been reported by Husain *et al.*[42], Manafi *et al.*[43] and Sikandar *et al.*[44], who found that the probiotic led to an increase in feed efficiency and growth performance. Regarding egg mass, there was a significant increase over several months of mass egg production in ducks that received feed with probiotics and the ducks also showed greater resistance to diseases affecting the digestive system[45]. Conversely, some researchers have reported[40,27,28] that probiotics in feed did not significantly influence production or feed efficiency. Inconsistent results from probiotic supplementation may be due to differences in the number of suitable live bacteria, animal age and supplementation strains[46].

Saccharomyces spp. supplementation in duck feed did not significantly influence egg production. This result is similar to the findings of Davis and Anderson[46], who reported that there was no increase in egg production in laying hens given probiotic bacteria, including *Lactobacillus* and *Bacillus*. This finding is also in accordance with Kalavathy *et al.*[47], who reported that probiotic supplementation had no significant effect on egg production in chickens. Egg production in hens provided with bacterial and fungal probiotics was not significantly different from that in control chickens[48]. Probiotic supplementation with *Saccharomyces* spp. Kb-5 in laying ducks can significantly improve the external egg quality characteristics (eggshell, egg yolk, skin thickness and Ca content) of laying ducks. The beneficial effects on the thickness and strength of the eggshell observed in this study are directly related to the reduction in the number of damaged eggs. Increased egg quality due to probiotic supplementation was reported by Saleh *et al.*[49], who found that diets supplemented with probiotics (*Aspergillus awamori* and lactic acid bacteria) showed the potential to increase egg weight, feed efficiency and eggshell quality during the first laying phase. Similar significant results in egg weight and eggshell quality were also obtained from chicken feed with a mixture of probiotic content[47,50].

Tang *et al.*[10] and Mahdavi *et al.*[51] reported that probiotics (*B. amyloliquefaciens*) showed a positive effect on egg production and the quality of eggshells. The positive effects of probiotic supplementation on the quality characteristics of eggshells have been reported by Abdelqader *et al.*[1] and Li *et al.*[52]. Swiatkiewicz *et al.*[53]. related the positive effects of probiotics on eggshell quality parameters due to an increase in the availability of Ca minerals in the intestine. However, the addition of probiotics does not have the expected significant effect on shell hardness or shell thickness[54]. Although, the increase in albumen quality was not significant, no reasonable explanation could be offered for the improvement in the albumen quality in the microbial additive group. Mahdavi *et al.*[51]. and Mohebbifar *et al.*[55] did not find a significant effect of the inclusion of dietary probiotics on egg quality. Fathi *et al.*[27] observed beneficial effects of probiotic supplementation on the quality of eggshells but no effects on the internal egg quality properties. Dimcho *et al.*[56] found that probiotic inclusion did not affect the total serum protein concentration of chicken eggs. Additionally, Alkhalf *et al.*[57] reported that total protein in serum and in albumin was not significantly affected by dietary probiotic supplementation. Sun *et al.*[50] reported that dietary supplementation with red yeast rice can improve the egg quality of laying hens without affecting egg production performance. In addition, An *et al.*[35] found that levels of triglycerides, aspartate aminotransferase, alanine aminotransferase and glucose in serum were reduced in the probiotic group compared to those in the control group. Similar results have been observed with certain types of probiotics[39,58]. Saleh *et al.*[49] reported that the concentrations of Ca, P and Zn in egg yolks were increased by probiotics (*Aspergillus awamori* and lactic acid bacteria). Similarly, the results obtained in the previous experiments[5,24,25] showed an increase in Ca, Co, P and Mn retention in chickens given probiotic diets.

Cholesterol levels of egg yolks were significantly lower in ducks fed diets containing *Saccharomyces* spp. (Groups S2 and S3). Some researchers[50,59,60] have reported that manipulation of enteric microflora with probiotics plays an important role in altering chicken lipid metabolism and can reduce cholesterol levels in egg yolks and serum. According to Alkhalf *et al.*[3], probiotics can reduce cholesterol levels in the blood by deconjugating bile salts in the intestine, thus preventing them from acting as precursors in cholesterol synthesis. *Saccharomyces* spp. is also found to have a high hydrolytic activity on bile salts, which leads to the deconjugation of bile salts[15]. The reduction in cholesterol circulation with additional yeast (*S. cerevisiae*) is very unusual and is in accordance with the results of other researchers. According to Istiqomah *et al.*[26], *S. cerevisiae* has the potential to assimilate cholesterol in vitro. Plasma cholesterol and triglycerides are reduced in laying hens fed probiotics[27]. Paryad and Mahmoudi[61] also reported that adding 1.5% *S. cerevisiae* to the diet could reduce serum cholesterol in broiler chickens. Decreased blood cholesterol levels due to probiotic inclusion have also been reported in broilers fed probiotics and in layers supplemented with probiotics[62]. The administration of *Lactococcus lactis* can reduce meat cholesterol levels in breast and thigh meat in chickens[63]. It is possible that the efficacy of probiotics depends on several factors including the composition of microbial species (single or multistrain mixture), viability, supplementation level, application method, application frequency, overall diet, bird age, overall agricultural cleanliness and environmental pressure factors[64,65]. There is no clear mechanism that has been reported to be responsible for reducing lipid synthesis by prebiotics and probiotics. The reduction may be due in part to an increase in beneficial bacteria such as *Lactobacillus*, which decreases the activity of acetyl-CoA carboxylase, a rate-limiting enzyme in fatty acid synthesis[28]. Conversely, An *et al.*[66] and Kalavathy *et al.*[67] reported that there were no significant differences in serum enzyme activity or concentrations of various cholesterol fractions as a result of probiotic supplementation. According to Chen *et al.*[21], inconsistent results from probiotic supplementation are probably due to differences in the number of suitable live bacteria, animal age and supplementation strains. This result is the same as that reported by Kang *et al.*[68], showing no significant changes in the lipid profile of mice given the probiotic *Lactobacillus gasseri* and showing the hypocholesterolemic effects of several strains of bacteria, including *Lactobacillus acidophilus*[69]. However, Tang *et al.*[60] reported that laying hens given probiotics showed significant reductions in the total saturated fatty acids in egg yolks compared to controls. Supplementation with probiotics *Aspergillus awamori* and lactic acid bacteria modified the fatty acid profile of egg yolk by increasing unsaturated fatty acids and reducing saturated fatty acids[49].

CONCLUSION

We conclude that supplementation with 0.20-0.30% probiotics *Saccharomyces* spp. Kb-5 in laying duck diets increased egg mass, feed efficiency, feed digestibility, yolk color, yolk and egg shell weight, shell thickness and Ca content in the yolk and eggshell but decreased yolk cholesterol contents.

SIGNIFICANCE STATEMENT

This study discovered information about the potential effect of *Saccharomyces* spp. Kb-5 isolated from buffalo rumen fluid as a probiotic on egg production and egg quality of laying ducks. Therefore, a new theory about optimal inclusion rates of *Saccharomyces* spp. Kb-5 isolated from buffalo rumen in rice bran-based rations may be useful for further research.

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