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Effects of Dietary Probiotics on Performance, Egg Quality and Yolk/Serum Cholesterol of Laying Hens

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Abstract: In an experiment with 112 laying hens from a commercial strain (Hy-line W36), the effects of probiotics Biosaf SC 47 (SC) containing a minimum of 5×10^9 colony forming units (cfu) g^{-1} *Saccharomyces cerevisiae* (strain NCYC Sc 47) and BioPlus 2B (BP) containing a minimum of 3.2×10^9 cfu g^{-1} of *Bacillus licheniformis* (strain CH200) and *B. subtilis* (strain CH201) on performance, egg quality and yolk/serum cholesterol was determined. In the 10 week experiment, hens (46 to 55 weeks of age) were allocated to seven dietary treatments being: SCBP0, SC200, SC300, SC400, BP400, BP800 and BP1200 of 16 birds each and received a diet *ad libitum*. The diet for SCBP0 treatment had no probiotic (control), while those for treatments SC200, SC300 and SC400 included 200, 300 and 400 g t^{-1} of probiotic SC, respectively and those for treatments BP400, BP800 and BP1200 included 400, 800 and 1200 g t^{-1} of probiotic BP, respectively. Inclusion of the probiotics SC at levels up to 400 g t^{-1} and BP at levels up to 1200 g t^{-1} in diets of laying hens resulted in similar traits, relative to final BW, DFC, egg weight, egg mass, egg shell breaking strength, haugh units, albumen index, yolk color, and yolk and serum cholesterol. Egg production and egg shell weight did not differ ($p>0.05$) between treatment SCBP0 and all other treatments, but egg production was higher ($p<0.05$) in treatment SC400 compared to treatment BP1200 and egg shell weight was higher ($p<0.05$) in treatments SC300, SC400, BP800 and BP1200 compared to treatment BP400. Feed efficiency was higher ($p<0.05$) in treatments SC200, SC300 and BP800 compared to treatment SCBP0 and egg shell thickness was higher ($p<0.05$) in treatment SC200 compared to treatments SCBP0 and BP400, whilst no other differences were observed among treatments. No deaths occurred during the experiment.

Key words: Laying hens, *Saccharomyces cerevisiae*, *bacillus licheniformis*, *B. subtilis*, performance, egg quality, yolk/serum cholesterol

INTRODUCTION

It has long been known that a well-balanced intestinal flora bars the way to pathogens trying to enter the body. Approximately 90% of the intestinal flora of birds is composed by facultative bacteria, while the remaining 10% consists of *Escherichia coli*, *Clostridium*, *Staphylococcus*, *Pseudomonas* and others[1]. Products, including antibiotics, which improve poultry production through intestinal flora manipulation, have been used for decades. In recent years, probiotics, being considered "natural" products, have received increasing attention. Probiotics are live microbial feed supplements that beneficially affect the health and well-being of the host animal by improving its gastrointestinal microbial balance[2]. The majority of probiotic products are based on *Lactobacillus* sp., *Streptococcus* sp. and *Bacillus* sp., although microscopic fungi, including *Saccharomyces* yeasts, are also used[3]. Probiotic inclusion in feeds benefit the host animal by changing the gut microflora, producing antibiotics, synthesizing lactic acid with consequent reduction in intestinal pH, colonizing the intestinal mucosa, preventing toxicosis and stimulating immune responses in the gut[3].

In poultry, the dietary inclusion of probiotics improved egg size, egg production and egg quality[4] and the supplementation of a *Lactobacillus* culture in a maize-barley based diet improved feed efficiency, egg weight, egg production and albumen quality[5]. Furthermore Mahdavi *et al.*[6], reported that inclusion of the probiotic BioPlus 2B in laying hens caused no significant decrease in feed consumption, egg production and egg weight. It has also been observed that yolk and serum cholesterol concentration can be suppressed by probiotics[4,6-8]. However, to our knowledge there is no published information on effects of *Saccharomyces cerevisiae* on egg production and quality, and yolk/serum cholesterol concentration of laying hens. Thus, the

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objective of this study was to evaluate the use of *S. cerevisiae* and a combination of *Bacillus licheniformis* and *B. subtilis* in diets of laying hens, relative to performance, egg quality and yolk/serum cholesterol concentration.

MATERIALS AND METHODS

Probiotics: The two probiotics used in this study were obtained from commercial processors. The first probiotic Biosaf SC 47 (SC) containing a minimum of 5×10^9 colony forming units (cfu) g^{-1} *S. cerevisiae* (strain NCYC Sc 47) was obtained from Société Industrielle Lesaffre (France), whilst the second probiotic BioPlus 2B (BP) containing a minimum of 3.2×10^9 cfu g^{-1} of *B. licheniformis* (strain CH200) and *B. subtilis* (strain CH201) was obtained from Chr. Hansen A/S (Denmark).

Laying hens: One hundred and twelve laying hens from a commercial strain (Hy-line W36, Hy-line Company, Urmia, Iran) were randomly allocated to seven dietary treatments (SCBP0, SC200, SC300, SC400, BP400, BP800 and BP1200) in a completely randomized design. All birds used in the experiment were cared for according to applicable recommendations of U.S National Research Council[9]. Hens of each treatment were divided into four subgroups (replicates) of 4 birds each and accommodated to 4 floor pens/treatment. At the beginning of the experiment, hens of all treatments had similar body weight (BW, 1373±28 g), egg production (0.86±0.02 eggs/hen/day) and egg weight (52.34±2.82 g). All 48 pens were identical and were equipped with similar troughs for diets and water. During the 10 week experimental period, from 46 to 55 week of age, all hens in the seven treatments received the same basal diet Table 1, according to nutrient requirements of laying hens as given by NRC[10]. Treatment SCBP0 was not supplemented with any probiotic (control). The probiotic SC was incorporated in the diet of SC200, SC300 and SC400 treatments at levels of 200, 300 and 400 g t^{-1}, respectively, and the probiotic BP was incorporated in the diet of BP400, BP800 and BP1200 treatments at levels of 400, 800 and 1200 g t^{-1}, respectively.

During the experimental period, conventional management procedures were employed, natural and artificial light was provided for 17 h per day, ambient temperature was controlled and birds were fed and watered *ad libitum*. Hens' BW (g) was measured at the start and end of the study on a pen basis. Mortality, feed consumption (DFC, g), egg production (eggs/hen/day), egg weight (g), egg mass (g/hen/day) and feed efficiency (kg feed/kg egg mass) were recorded daily. Furthermore, in 2 eggs per replicate collected randomly at the 10th week of the experimental period, egg quality was evaluated by measuring egg shell weight (after washing and drying overnight at 80°C and then weighed with a digital balance), egg shell thickness[11], egg shell breaking strength[12], Haugh units[13], albumen index (albumen height/albumen diameter ×100), yolk color (by using a Roche fan) and yolk cholesterol. At the 10th week of the experimental period, blood samples (2 mL) were obtained from 8 hens, randomly selected from each treatment (2 from each subgroup), for cholesterol analysis from the brachial vein into vacuum tubes with no additive contained; then centrifuged at 3000 rpm for 15 min to obtain blood serum[5] and stored at -22°C until analysis. Yolk and serum cholesterol levels were determined by the enzymatic chromatometric method CHOD-PAP[14], using a spectrophotometer CECIL (serial no 71055, England) for yolk cholesterol and an autoanalyzer COBAF-MIRA model L (serial no 26-56-59, Sweden) for serum cholesterol.

Table 1: Composition[1] of laying hen basal diet (as fed basis)

	Basal Diet
Ingredient composition (kg t^{-1})	
Corn grain, ground	592.33
Wheat grain, ground	58.2
Wheat bran	60.2
Soybean meal (440 g kg^{-1} CP)	168
Fish meal (642 g kg^{-1} CP)	20
Oyster shell	74.7
Vegetable oil	5
DL-Methionine 990 g kg^{-1}	0.37
Dicalcium phosphate	12.4
Salt	3.8
Vitamin premix[2]	2.5
Mineral premix[3]	2.5
Chemical composition[4] (g kg^{-1})	
Metabolizable energy (kcal kg^{-1})	2700
Crude protein	155
Crude fat	32.6
Crude fiber	28.9
Calcium	32.5
Avail. Phosphorus	4.0
Sodium	1.8
Linoleic acid	15.5
Arginine	9.2
Lysine	7.6
Methionine + cystine	5.8
Threonine	5.7
Tryptophan	1.9

[1]Dry matter content 900 g kg^{-1}. [2] Premix supplied per kg of diet: 9000 IU vitamin A, 1.78 mg vitamin B_1, 6.6 mg vitamin B_2, 30 mg niacin, 10 mg pantothenic acid, 3 mg vitamin B_6, 0.15 mg biotin, 1500 mg choline, 0.015 mg vitamin B_{12}, 2000 IU vitamin D, 18 IU vitamin E, 2 mg vitamin K_3. [3]Premix supplied per kg of diet: 10 mg Cu, 0.99 mg I, 50 mg Fe, 100 mg Mn, 0.08 mg Se, 100 mg Zn. [4] All values were calculated from NRC values (1994)

Statistical Analysis: Performance, egg quality and serum/yolk cholesterol concentration of laying hens were statistically analyzed by one-way analysis of variance with the pen of hens being the experimental unit, while significant differences among treatment means were tested using the Duncan's test at the 5% probability level[15]. The statistical analysis was made with the help of the SPSS Staatistical Software Package[16].

RESULTS AND DISCUSSION

Effects of dietary probiotics on various egg production, egg quality and yolk/serum cholesterol of laying hens are presented in Table 2. At the end of the experiment, final BW, DFC, egg weight, egg mass, egg shell breaking strength, haugh units, albumen index, yolk color and yolk and serum cholesterol were similar among all treatments. Additionally, egg production and egg shell weight did not differ ($p>0.05$) between treatment SCBP0 and all other treatments. However, egg production was higher ($p<0.05$) in treatment SC400 compared to treatment BP1200 and egg shell weight was higher ($p<0.05$) in treatments SC300, SC400, BP800 and BP1200 compared to treatment BP400. Feed efficiency was higher ($p<0.05$) in treatments SC200, SC300 and BP800 compared to treatment SCBP0 and egg shell thickness was higher ($p<0.05$) in treatment SC200 compared to treatments SCBP0 and BP400, whilst no other differences were observed among treatments. Finally, no deaths occurred during the experiment.

Our results are in agreement with Nahashon *et al.*[17] who reported that feed efficiency for hens fed a diet having no *Lactobacillus* supplementation was significantly lower than the hens fed diets supplemented with *Lactobacillus* at various levels 0, 1100 and 2200 ppm for 24 weeks. In contrast Belavi *et al.*[18], found that feed efficiency was lower ($p<0.05$) in the group of hens that consumed 500 g t^{-1} of commercial probiotic (Protexin™) compared to the unsupplemented group. MoreoverJin *et al.*[19], showed that the *Lactobacillus* fed to broilers did not alter feed efficiency on day 21, but improved it on day 42. In addition, Mahdavi *et al.*[6] did not find any significant difference on production characteristics when laying hens were fed the probiotic BP at inclusion levels up to 2000 g t^{-1} of their diet. In another 3 experiments which were conducted in 3 different geographical locations Miles *et al.*[20], reported that the *Lactobacillus* culture incorporation at levels 125, 375 and 625 g t^{-1} of the laying hens' diet gave equivocal results. Feeding the *Lactobacillus* culture resulted in increased egg production in the first experiment, a numerical improvement in the second experiment and no difference in the third experiment. In contrast[4], reported that egg production and feed efficiency increased ($p<0.05$) when *L. acidophilus* was supplemented (4×10^6 cfu g^{-1}) in hens' diet compared to the unsupplemented diet. Furthermore, the supplementation with 100 g t^{-1} of a commercial probiotic (Probiolac) in hens' diet improved the daily egg production during the declining phase of layers compared to the unsupplemented diet and to the diet supplemented with 150 g t^{-1} of this probiotic[21].

Direct-fed microbial benefit the host animal by stimulating appetite[20,22]. In this study, the numerically increased feed consumption may be associated with the stimulation appetite in treatment SC400 and consequent improved on egg production, egg mass and egg weight. Fundamentally, concerning the mechanism by which probiotics affect poultry performance, it is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients[23].

The diverse findings in studies with probiotics in laying hens' diets could be explained by a possible increase in gut motility which may occur in the presence of excessive numbers of microorganisms, thereby altering nutrient availability for absorption at desired points[24]. Other beneficial, already existing in the gut, bacterial

Table 2: Performance, egg quality and yolk/serum cholesterol of laying hens

	Treatment[1]							
	SCBP0	SC200	SC300	SC400	BP400	BP800	BP1200	SEM
Final body weight (g)	146	21476	1418	1458	1440	1416	1530	50.011
Daily feed consumption (g)	103.25	106.58	103.65	107.21	99.87	103.62	100.84	2.373
Egg production (eggs/hen/day)	0.857^{ab}	0.841^{ab}	0.836^{ab}	0.877^{a}	0.823^{ab}	0.815^{ab}	0.802^{b}	0.221
Egg weight (g)	61.11	58.80	59.62	61.40	59.22	60.43	59.70	0.707
Egg mass (g/hen/day)	52.39	49.52	49.84	52.95	48.75	49.31	47.90	1.595
Feed efficiency (g feed g^{-1} egg mass)	1.96^{b}	2.18^{a}	2.15^{a}	2.03^{ab}	2.07^{ab}	2.14^{a}	2.13^{ab}	0.057
Egg shell weight (g)	5.38^{ab}	5.43^{ab}	5.58^{a}	5.72^{a}	5.09^{b}	5.56^{a}	5.77^{a}	0.126
Egg shell thickness (mm)	0.273^{b}	0.289^{a}	0.279^{ab}	0.277^{ab}	0.266^{b}	0.277^{ab}	0.279^{ab}	0.005
Egg shell breaking strength (kg cm^{-2})	2.458	2.512	2.594	2.709	2.515	2.615	2.840	0.157
Haugh units	84.02	81.82	84.08	85.68	82.41	84.89	81.35	1.930
Albumen index	7.32	6.67	7.33	7.30	6.83	7.24	6.85	0.290
Yolk color	4.33	4.42	4.17	4.25	4.15	4.17	4.42	0.159
Yolk cholesterol (mg g^{-1})	14.0	13.1	13.1	12.7	13.0	12.5	13.2	0.599
Serum cholesterol (mg100^{-1} mL)	179.97	204.96	179.12	164.13	199.13	149.97	192.47	21.573

[1]SCBP0 = control treatment, SC200 = treatment with 200 g t^{-1} SC, SC300 = treatment with 300 g t^{-1}SC, SC400 = treatment with 400 g t^{-1}SC, BP400 = treatment with 400 g t^{-1} BP, BP800 = treatment with 800 g t^{-1}BP, BP1200 = treatment with 1200 g t^{-1}BP. [a-b]Means within each row with different superscripts are significantly different.

populations may also be related, disrupting cohabitation of the established micro flora[20]. Moreover Siriwar [25], found that *Lactobacillus* cultures are host-specific. Perhaps the probiotics strains used in studies with poultry were not host specific or well adapted for avian used in the experiment. Generally, it may be concluded that the different results of performance obtained in this study, relate to the strain of microorganism, its genus, concentration and the form of bacteria used (viability, dryness and spore forming or their products).

Concerning egg quality Nahashon *et al*.[17,22,26], showed that the decrease of pH in gastrointestinal tract of layers due to probiotic dietary supplementation resulted in an increase in calcium retention and improvement in shell characters. In addition Robinson[27], suggested that lactic acid produced by *Lactobacilli* might encourage better absorption of calcium and phosphorus from the digestive tract. Moreover Mohan *et al*.[7], showed a slight improvement in egg shell thickness in hens supplemented with probiotic for 10 weeks during the peak period, but Tortuero and Fernandez[5], found that egg shell calcium was not affected by the addition of *Streptococcus faecium* to the diet of laying hens. Furthermore, Haugh unit values and yolk color were not affected by feeding laying hens with *L. acidophilus* culture at three geographical locations[20] and with humate (a part of fertilizer that is derived from plant matter decomposed by bacteria[28] and probiotic (Protexin™) during the late laying period[29]. In fact, Haugh unit and albumen index are two major indicators to evaluate egg quality and do not change by dietary regiment, but by aging[30].

Concerning yolk/serum cholesterol Haddadin *et al*.[4], reported that dietary inclusion of *L. acidophilus* in three ages (40, 44 and 48 weeks) affects yolk cholesterol in 40 week of production, but not in 44 and 48 and Mahdavi *et al*.[6], who worked on 28-39 week old layers, obtained significant decrease in egg yolk and plasma cholesterol concentration of hens due to BP inclusion. However, the variation in yolk/serum cholesterol concentration may attributed to numerous factors. Elkin and Yan[31] reported that cholesterol content in the eggs is influenced by genetic factors, diet composition, lay intensity, layer age and medical treatment. Mott *et al*.[32] observed that *Lactobacilli* could have been absorbing the cholesterol in the gastrointestinal tract, thus keeping cholesterol from being absorbed by the body. Some researchers showed that the addition of probiotics reduced the plasma cholesterol and triglyceride significantly[4,6-8], confirming the important roles of gastrointestinal tract microorganisms in recycling of lipids. It is reported that probiotic such as *Lactobacilli* can assimilate cholesterol[33] and deconjugated bile acids [34] and this leads to a reduction in serum cholesterol levels. The correlation coefficient between serum and yolk cholesterol has not been confirmed[35,36], however Haddadin *et al*.[4], Hargis[37] and Ghazalah *et al*.[38] suggested that this link could be expected between serum and egg lipid. In addition, Hollands *et al*.[39] reported that chickens selected for low serum cholesterol also had lower yolk cholesterol, but Weiss and Scott[40] concluded that ovarian synthesis of cholesterol is responsible for maintaining the level of cholesterol in the egg and that serum cholesterol level has little effect on egg cholesterol levels. Marks and Washburn[36] suggested an alternative hypothesis that unidentified environmental factors or interactions maybe responsible for the conflicting reports regarding this relationship as well.

CONCLUSION

Inclusion of probiotics Biosaf SC 47 (*Saccharomyces cerevisiae*, strain NCYC Sc 47) at levels up to 400 g/t and BioPlus 2B (*Bacillus licheniformis*, strain CH200, and *B. subtilis*, strain CH201) at levels up to 1200 g/t did not, substantially, affect performance, egg quality and yolk/serum cholesterol of laying hens (Hy-line W36) from 46 to 55 weeks of age. Further research is needed to supply higher levels of these probiotics in younger aged layers to test the hypothesis that Biosaf SC 47 and BioPlus 2B dietary inclusion beneficially affects performance, egg quality and yolk/serum cholesterol of hens.

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