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Effect of *Saccharomyces Cerevisiae* and Bioplus 2B on Egg Quality and Yolk/Serum Cholesterol of Laying Hens

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***Abstract* – In an study include 120 laying hens from a commercial strain (Hy-line W36) the effect of dietary probiotic Biosaf SC 47 (SC) containing a minimum of 5×10^{10} colony forming units (cfu)gr^{-1} *Saccharomyces cerevisiae* (strain NCYC sc 47) and Boiplus 2B(BP) containing a minimum of 3.2×10^{9} (cfu)gr^{-1} *Bacillus subtilis*(CH201) and *Bacillus lichenioformis* (CH200) on egg quality and yolk serum cholesterol was determined. In 10 week experiment, hens (46 to 55 weeks of age) were allocated to three dietary treatments being control, SC, BP. Treatment SCBP0 had no probiotic (control) while those for treatments SC, BP included 200,300,400g t^{-1} of *S. cerevisiae* and 400,800,1200 gr t^{-1} Bioplus 2B respectively. Using the different probiotics caused significant increase ($p<0.05$) egg shell weight, egg shell breaking and egg shell thickness but it had no significant effects on other traits.**

***Keywords* – Laying Hens, *Saccharomyces Cerevisiae, Bacillus Licheniformis, B. Subtilis,* Egg Quality, Yolk/Serum Cholesterol.**

I. INTRODUCTION

Probiotics are used to improve intestinal health and to stimulate the immune system. Approximately %90 of the intestinal flora of birds is composed by facultative bacteria. The remaining %10 consists of *E.coli*, *Clostridiums*, *Staphylococcus*, *Pseudomonas* and others (Fox, 1988). The U.S national food Ingredient Association defines probiotic (direct feed microbial) as a source of live naturally occurring microorganisms, and this include bacteria, fungi and yeast (miles and Bootwalla, 1991). Supplementation of probiotic (protexin) during the late laying period increased egg production, reduced mortality, and improved feed conversion efficiency but did not improve egg quality (Yuruket et al., 2004). Using the different levels of probiotic (Bioplus 2B) improved in Goblet cell numbers, apical cells of villus and reduced in plasma cholesterol, plasma triglyceride and egg cholesterol (Mahdavi et al., 2005). The aim of this examination was to determine the use of *S. cerevisiae* and Bioplus 2B in the diet of laying hens and to compare these two probiotic relative to yolk/serum cholesterol concentration and egg quality.

II. MATERIALS AND METHOD

Probiotics: The two probiotics used in this study were obtained from commertial processors. The first probiotic Biosaf SC 47 (SC) containing a minimum of 5×10^{10} colony forming units (cfu)gr^{-1} *saccharomyces cerevisiae* (strain NCYC sc 47) was obtained from the company of Société Industrielle LESAFFRE(France). Whilst the second probioic Bioplus 2B(BP) containing minimum of 3.2×10^{9} (cfu)gr^{-1} *Bacillus subtilis* (CH201) and *Bacillus lichenioformis* (CH200) was obtained from HansenA/S (denmark).

Laying hens: one hundred and twenty laying hens from a commercial strain(Hy-line W36, Hy-line company, Urmia, Iran) were randomly allocated to three dietary treatment s(control, SC, BP) completely randomized design. All birds used in the experiment were cared for according to applicable recommendations of U.S National Research Council (NRC, 1996). Hens of each treatment were divided in ten replications of 4 birds each.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 30 pens were identical and were equipped with similar through for diets and water. During 10 week experiment period from 46 to 55 week of age all hens the 3 treatments received the same basal diet Table 1, according to nutrient requirement of laying hens as given by National Research Council value (NRC, 1994).

Treatment SCBP not supplemented with any probiotic(control). Treatments SC and BP at the levels of 200, 300 and 400, g t^{-1} S. *cerevisiae* and400, 800 and 1200 g t^{-1} Biopluse 2B incorporated, respectively. Natural and artificial light was provided for 17h per day, ambient temperature was controlled and birds were fed and watered *ad libitum*. Two eggs from each cage were selected randomly to evaluate the egg quality at the end of trial. 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 (Lundholm, 1987) egg shell breaking strength (potts, 1974), Haugh units (Haugh, 1937), albumen index (albumen height/albumen diameter×100) and yolk color (by using a Roche fan). Yolk cholesterol and blood cholesterol was determined at the end of the trial. Blood samples were taken (2ml) from the brachial vein of two hens in each cage at the 10^{th} week of study, and centrifuged (3000 rpm for 15min) immediately and plasma collected (Tortuero et al., 1995). Plasma and yolk cholesterol was estimated by the Enzymatic, colorimetric CHOD/PAP (Siedel 1980) using a spectrophotometer model Cecil made in England (serial no 71055), for yolk cholesterol and autoanalyzer to determine plasma cholesterol made in Sweden model L, COBAF-MIRA model L (serial no 26-56-59, Sweden)

Statistical analysis: Egg quality parameters and yolk/serum cholesterol were statistically analyzed by one-way analysis of variance, the comparison was among the experimental groups and non orthogonal. The statistical

analysis was made with the help of the SPSS statistical software package (SPSS, 2003).

III. Result and Discussion

This study carried out to evaluate the effect of probiotic supplementation on egg quality and yolk/serum cholesterol. The results are presented in Tables 2 and 3. A statistically improvement was seen in shell characteristics after 10 week inclusion of probiotic at the diet of layers. At the end of experiment Hugh unit, Albumen index yolk color, yolk cholesterol were similar among all treatments ($p>0.05$). Egg shell weight and shell breaking strength was higher ($p<0.05$) in treatments SC, BP compared to treatment control. Egg shell thickness was higher ($p<0.05$) in treatment SC than treatment BP and PSC0.

Our results are in agreement with Mohan et al. (1995) who reported that inclusion of *lactobacillus acidophilus* at levels of 0, 100, 150 mg kg^{-1} fed in the diets of laying hens, marginally improved egg shell thickness. Furthermore, Nahashon et al. (1994a, b, 1992) showed that decrease of pH in gut tract of layers due to probiotic dietary supplementation resulted increase in calcium retention and improvement in shell characters, but Tortuero and Fernandez (1995) found that egg shell calcium was not affected by the addition of *streptococcus facium* to the diet of laying hen. More over, slight improvement was seen in egg shell thickness in hens diets supplemented with probiotic for 10 week during the peak period (Mohan et al., 1995). Miles et al. (1981) showed that neither Haugh unit values nor yolk color were not statistically affected by feeding live *lactobacillus acidophilus* culture at three geographical locations. At really, Haugh unit and albumen index are two major indicators to evaluate egg quality and do not change by dietary regiment but by aging (Silversides et al., 2001) Similarly, Tortuero et al. (1975) reported that serum cholesterol levels of cecectomized laying hens were higher than normal birds and they found that feeding *L. acidophilus* to laying hens (during 20 to23 month of age) resulted in a significant decrease in serum cholesterol. Mott et al. (1973) observed that *Lactobacilli* could have been absorbing the cholesterol in the gastrointestinal tract, so prevent cholesterol from being absorbed by the body. Some authors showed that the addition of probiotic reduced the plasma cholesterol and triglyceride significantly by assimilates cholesterol (Abdulrahim et al., 1996; haddadin et al., 1996; Mohan et al., 1995; Mahdavi et al., 2005). On the other hand, Sutton et al. (1984) reported that hens during the egg production need to cholesterol synthesis, thus blood cholesterol increase as they mature.

At the end of this examination results indicated that application of probiotics Biosaf SC 47 *Saccharomyces cerevisiae* (strain NCYC sc 47) at the levels up 300g t^{-1} and bioplus 2B *Bacillus lichenioformis*(CH200) *Bacillus subtilis*(CH201) at the levels up to 800 g t^{-1} numerically reduced yolk cholesterol and significantly increased shell parameters. Further research is needed to use another levels of these probiotics in different aged layer hens.

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Table 1: The ingredients and chemical composition[1] of basal diet (as fed basis)

Ingredients	%
Yellow corn	59.233
Soybean meal	16.8
Fish meal	2
Oyster shell	7.47
Wheat	5.82
Wheat bran	6.02
Dicalcium phosphate	1.24
Oil	0.5
Salt	0.38
Mineral premix[2]	0.25
Vitamin premix[3]	0.25
DL-Methionine	0.037
Total[4]	100
ME(kcal/g)	2.7
Protein%	15.5
Calcium%	3.25
Available phosphate%	0.4
Sodium%	0.18
Arginine %	0.92
Lysine%	0.76
Threonine %	0.57
Tryptophan %	0.19
Met+cys%	0.58

[1]Dry matter content 9000g/gr. [2]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. [3]Premix supplied per kg of diet: 9000Iu vitamin A,1.78 mg vitamin B_1, 6.6 mg vitamin B_2, 30 mg niacin, 10mg pantothonic acid, 3 mg vitaminB_6 0.15 mg biotin, 1500 mg choline, 0.015 mg vitamin B_{12}, 2000Iu vitamin D, 18 IU vitamin E, 2 mg vitamin k_3,[4] All value were calculated from NRC value (1994)

Table 2: Effect of probiotic on egg quality treats

treatment	Egg shell weight(g)	Egg shell breaking strength (kg $cm^{-2)}$	Haugh unit	Albumen index	Egg shell thickness(mm)	Yolk color
Control	5.35^a	2.458^a	84.02	7.32	0.273^a	4.33
SC	5.57^b	2.605^b	83.86	7.1	0.287^b	4.28
BP	5.47^b	2.650^b	82.88	6.97	$.274^a$	4.246
SEM	0.150	0.125	1.805	0.220	0.002	0.125

[a-b] Means within each row with different superscripts are significantly different.

Table 3: Cholesterol concentration of egg yolk and blood in experimental laying hens

treatment	Yolk cholesterol(mg $g^{-1)}$	Serum cholesterol(mg100^{-1} mL)
Control	14	179.97
SC	12.96	182.73
BP	12.9	180.52
SEM	0.788	25.256

[a-b] Means within each row with different superscripts are significantly different.