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Research Article

Effects of Dietary Inactive Yeast and Live Yeast on Performance, Egg Quality Traits, Some Blood Parameters and Antibody Production to SRBC of Laying Hens

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Abstract

This study was carried out to determine the effects of dietary inactive yeast and live yeast on performance, egg quality traits, some blood parameters and antibody production to sheep red blood cell (SRBC) of laying hens during 16 weeks. A total of 96 Hyline Brown laying hens were allocated into one control group and three treatment groups each containing 24 hens. Each group had six replicate groups of 4 hens. A basal diet was supplemented with 1 g/kg inactive yeast (yeast autolysate, InteWall, *Saccharomyces cerevisiae*), 0.5 g/kg live yeast (InteSacc, *Saccharomyces cerevisiae*) and 1 g/kg inactive yeast + 0.5 g/kg live yeast in the diets of the first, second and third treatment groups, respectively. At the end of the study the results indicated that dietary treatments did not affect feed intake, interior and exterior egg quality characteristics. Dietary inactive yeast supplementation improved hen-day egg production (P=0.024) and feed conversion ratio (P=0.017) and decreased egg yolk cholesterol concentration (P=0.013). Antibody titers against SRBC and blood serum parameters were not affected by dietary treatments. The significant interaction was found in egg yolk cholesterol concentration (P=0.032) between inactive yeast and live yeast. As a result dietary inactive yeast at the level of 1 g/kg had beneficial effects in laying performance and in low cholesterol-egg production.

Keywords: *Blood characteristics, Egg quality, Inactive yeast, Live yeast, Performance*

Yumurta Tavuğu Karma Yemlerine İnaktif Maya ve Canlı Maya İlavesinin Performans, Yumurta Kalite Özellikleri, Bazı Kan Parametreleri ve SRBC'ye Karşı Antikor Üretimi Üzerine Etkileri

Özet

Bu araştırma yumurta tavuğu karma yemlerine inaktif maya ve canlı maya ilavesinin performans, yumurta kalite özellikleri, bazı kan parametreleri ve koyun eritrositine karşı (SRBC) antikor üretimi üzerine etkilerini 16 hafta süreyle incelemek amacıyla yapılmıştır. Toplam 96 adet Hyline kahverengi yumurta tavuğu her biri 24 adet içeren bir kontrol grubu ve üç deneme grubuna ayrılmıştır. Gruplar her birinde 4 tavuk bulunan altı tekerrür grubu kapsayacak şekilde düzenlenmiştir. Bazal karma yeme 1 g/kg inaktif maya (maya otolizatı, InteWall, *Saccharomyces cerevisiae*), 0.5 g/kg canlı maya (InteSacc, *Saccharomyces cerevisiae*) ve 1 g/kg inaktif maya+0.5 g/kg canlı maya ilave edilerek sırasıyla birinci, ikinci ve üçüncü deneme grupları karma yemleri oluşturulmuştur. Deneme sonucunda gruplar arasında yem tüketimi ile iç ve dış yumurta kalite özellikleri bakımından farklılık gözlenmemiştir. Karma yeme inaktif maya ilavesi yumurta verimini (P=0.024) ve yemden yararlanma oranını (P=0.017) olumlu yönde etkilemiş ve yumurta kolesterol konsantrasyonunu ise (P=0.013) azaltmıştır. Gruplar arasında SRBC'ye karşı antikor üretimi ve kan serum parametreleri bakımından farklılık gözlenmemiştir. İnaktif maya ve canlı maya arasında yumurta sarısı kolesterol konsantrasyonu bakımından önemli interaksiyon (P=0.032) bulunmuştur. Sonuç olarak inaktif mayanın 1 g/kg düzeyinde karma yeme ilave edilmesinin yumurta performansı ve düşük kolesterollü yumurta üretiminde yararlı olacağı kanısına varılmıştır.

Anahtar sözcükler: *Canlı maya, İnaktif maya, Kan parametreleri, Performans, Yumurta kalitesi*

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INTRODUCTION

Yeast products have been used increasingly in poultry diets as a feed additive after the ban on the use of antibiotic growth promoters in the EU. Inactive yeast as a prebiotics and live yeast as a probiotics are very important in growth promotion and disease resistance for poultry nutrition. Moreover, the effects of probiotics or prebiotics on the performance of livestock are contradictory with the improvements in some feeding trials [1].

There are some reports about the usage of various yeast and yeast products such as inactive dry yeast, yeast culture, yeast autolysate, yeast cell wall and live yeast in the diets of laying hens on performance [2-8]. Yalçın et al.[5] reported that yeast autolysate as inactive yeast at the levels of 2, 3 and 4 g/kg had beneficial effects on performance, egg cholesterol content and humoral immune response. However Sacakli et al.[3] concluded that inactivated brewer's yeast *(Saccharomyces cerevisiae)* had no beneficial effect on production parameters of hens fed with optimal diets and reared under proper management conditions. Hassanein and Soliman [9] concluded that dietary live yeast *(Saccharomyces cerevisiae)* supplementation at 4 and 8 g/kg can enhance the productive performance and nutrient utilization via the inhibitory effect of yeast against pathogenic bacteria. Chumpawadee et al.[10] observed that cassava yeast as probiotic source had positive effect on egg weight and egg shell thickness but had negative effect on egg production. Similarly, Dizaji and Pirmohammadi [11] reported that addition of yeast products to diets decreased egg production in laying hens. In the study of Ayasan et al.[12] dietary probiotic supplementation did not affect feed intake, feed conversion efficiency, egg weight, egg shell thickness and egg shape index but affected egg production and egg weight. However, as far as we know, there is no published report on the interaction of dietary inactive yeast and live yeast in laying hens. It was hypothesized that these two feed additives given in combination might enhance performance, egg traits and immune system.

Therefore the purpose of this study was to examine the effects of the dietary inactive yeast and live yeast on performance, egg quality traits, some blood characteristics and antibody titers to SRBC in laying hens.

MATERIAL and METHODS

Animals and Diets

A total of 96 Hyline Brown laying hens aged 54 wk were randomly assigned to one control group and three treatment groups each containing six replicate groups of 4 hens. They were housed in cages (30 cm x 44 cm x 44 cm) in a windowed poultry house with a 16/8 h light/dark regimen. Feed in mash form and water were provided *ad libitum* during the 16 wk experimental period. The diet was formulated to meet or exceed the nutrient requirements for Hyline Brown commercial layers [13]. The ingredients and chemical composition of the basal diet are shown in *Table 1*. The basal diet was supplemented with 1 g/kg inactive yeast, 0.5 g/kg live yeast and 1 g/kg inactive yeast + 0.5 g/kg live yeast in the diets of the first, second and third treatment groups, respectively. Inactive yeast (yeast autolysate, *Saccharomyces cerevisiae*, InteWall) and live yeast (InteSacc, *Saccharomyces cerevisiae*, $1x10^9$ cfu/g) derived from baker's yeast were obtained from Integro Food and Feed Manufacturing Company (İstanbul-Turkey). All animal use protocols were in accordance with the Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the protection of animals used for scientific purposes [14]. This study was conducted by the researchers based on protocols by Ankara University Ethical Commission Report (No: 2008/18/72).

Measurements, Sample Collection and Laboratory Analysis

Nutrient composition of basal diet were determined according to the AOAC [15]. The samples were ashed in a muffle furnace prior to the analysis of calcium and total phosphorus [16,17]. Metabolizable energy levels of samples were estimated using the Carpenter and Clegg's equation [18].

Hens were observed daily for evaluating mortality during the experiment. Eggs were collected daily and egg production was expressed on a hen-day basis. All the eggs laid during the last two consecutive days of every week were collected and weighed individually to determine the egg weight. Feed intake was recorded biweekly and calculated as g per day per hen. The feed conversion ratio was calculated as g feed per g egg.

Table 1. *Ingredients and chemical composition of the basal diets*
Tablo 1. *Bazal karma yemlerin yapısı ve kimyasal bileşimi*

Ingredients (g/kg)		Chemical Composition (Analyzed)	
Corn	615.5	Metabolizable energy[b] (kcal/kg)	2750
Soybean meal, 44% CP	215.5	Crude protein (g/kg)	167.0
Full fat soya, 38% CP	50.0	Calcium (g/kg)	40.6
Limestone	95.0	Total phosphorus (g/kg)	6.2
Dicalcium phosphate	17.0		
Salt	2.5		
DL-Methionine	2.0		
Vitamin mineral premix[a]	2.5		

[a] Supplied the following per kilogram of diet: 12.000 IU vitamin A, 2.400 IU vitamin D_3, 30 mg vitamin E, 2.5 mg vitamin K_3, 2.5 mg vitamin B_1, 6 mg vitamin B_2, 4 mg vitamin B_6, 20 mg vitamin B_{12}, 25 mg niacin, 8 mg calcium-D-panthotenate, 1 mg folic acid, 50 mg vitamin C, 50 mg D-biotin, 150 mg choline chloride, 1.5 mg canthaxanthin, 0.5 mg apo carotenoic acid esther, 80 mg Mn, 60 mg Zn, 60 mg Fe, 5 mg Cu, 1 mg I, 0.5 mg Co, 0.15 mg Se; [b]Metabolizable energy content of diets was estimated according to the equation of Carpenter and Clegg [18]

To determine the egg internal and shell quality characteristics, 120 eggs laid at 09:00 to 12:00 h were collected randomly from each group (20 eggs from each replicate in total) during four consecutive days of last two weeks. Each egg was weighed and their shape index, shell breaking strength and shell thickness were measured. Then yolk height, albumen height, yolk width, albumen width and albumen length were determined. By using these values, yolk index, albumen index and Haugh units were calculated as shown with Yalçın et al.[6]. Egg internal and external quality analysis were completed within 24 h of the eggs being collected [6]. Egg quality evaluation was performed for individual eggs, as it was done in relation to egg weight.

At the end of the experiment, 18 eggs per each group (3 eggs from each replicate) were randomly chosen to determine yolk cholesterol. Eggs were boiled for 5 min. Egg yolk was blended with isopropyl alcohol with a volume of 10 ml per g of yolk [19]. Cholesterol content of this extract was determined according to the enzymatic method of TECO [20]. Yolk cholesterol was calculated and expressed as mg per g yolk.

At the 13th wk of the experiment, all hens were injected with 0.1 ml of 0.25% suspension of sheep erythrocytes (SRBC) in phosphate buffer saline. Circulating anti-SRBC antibody titers were determined by the microhem-agglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the the serum dilution [21].

Blood samples were collected from vena brachialis under the wing from all fed hens at the end of the experiment and centrifuged at 3.000 x *g* for 10 min. Serum was collected and stored at -20°C for determination of total protein, albumin, uric acid, triglyceride, cholesterol and levels of aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) by Vitros 350 autoanalyzer (New York, USA; Product code 680-2153) using their accompanying commercial kits [22].

Statistical Analysis

Statistical analysis were done using SPSS program (SPSS Inc., Chicago, IL, USA). The experimental unit was the cage (n=6). The normality of data distribution was checked using the Kolmogorov-Smirnov test. The effects of inactive yeast and live yeast were examined by two-way ANOVA. Values were reported as means ± SEM. When interaction between inactive yeast and live yeast was detected, one-way ANOVA with Duncan test was used to determine the differences among the groups [23]. Level of significance was taken as P<0.05.

RESULTS

The effects of dietary inactive yeast and live yeast on laying performance are shown in *Table 2*. Dietary treatments did not significantly affect feed intake and egg weight. However hen-day egg production (P=0.024) and feed conversion ratio (P=0.017) was improved by inactive yeast supplementation. No interactions were seen between inactive yeast and live yeast in feed intake, egg production and feed conversion ratio. No mortality was seen during the 16 wk experimental period.

The inclusion of inactive yeast or live yeast in the diet of laying hens had no significant effect (P>0.05) on the values of internal and external egg quality characteristics *(Table 3)*.

Table 2. *The effects of dietary supplementation of inactive yeast and live yeast on performance characteristics in laying hens*
Tablo 2. *Karma yemlere inaktif maya ve canlı maya ilavesinin yumurtacı tavuklarda performans ölçütleri üzerine etkileri*

Inactive Yeast (g/kg)	Live Yeast (g/kg)	Feed Intake (g/day per hen)	Hen-day Egg Production (%)	Egg Weight (g)	Feed Conversion Ratio (g feed/g egg)
0		112.7	88.20^b	66.12	1.94^a
1		113.4	91.39^a	67.03	1.85^b
	0	113.2	88.71	66.80	1.91
	0.5	112.9	90.88	66.40	1.88
0	0	112.8	86.39	66.04	1.98
1	0	113.5	91.03	67.47	1.85
0	0.5	112.5	90.01	66.20	1.89
1	0.5	113.3	91.74	66.58	1.86
SEM		0.9	1.31	0.53	0.03
Two way ANOVA (P values)					
Inactive yeast		0.413	0.024	0.102	0.017
Live yeast		0.778	0.113	0.495	0.225
Inactive yeast X Live yeast		0.936	0.279	0.334	0.158

$^{a-b}$ *Means results within columns with different letters are significantly different (P<0.05); n = 6*

There were no interactions in these egg characteristics between inactive yeast and live yeast. Dietary inactive yeast supplementation decreased egg yolk cholesterol concentration (P=0.013). The interaction in egg yolk cholesterol content (P=0.032) between inactive yeast and live yeast was also observed.

Dietary supplementation of inactive yeast and live yeast did not affect blood serum parameters and antibody titers against SRBC *(Table 4)*. No interactions were also seen in these values.

DISCUSSION

Dietary inactive yeast supplementation improved hen-day egg production (P=0.024) and feed conversion ratio (P=0.017) and did not significantly affect feed intake and egg weight. However feed intake, hen-day egg production, egg weight and feed conversion ratio were not affected by live yeast inclusion. Some researchers also reported that inactive yeast [3,5,6,24,25] and live yeast [8,10,25,26] had no effect on feed intake of hens. Similarly, some researchers observed

Table 3. *The effects of dietary supplementation of inactive yeast and live yeast on egg quality characteristics and egg yolk cholesterol concentrations in laying hens*

Tablo 3. *Karma yemlere inaktif maya ve canlı maya ilavesinin yumurtacı tavuklarda yumurta kalite özellikleri ve yumurta sarısı kolesterol konsantrasyonu üzerine etkileri*

Inactive Yeast (g/kg)	Live Yeast (g/kg)	Shape Index (%)	Breaking Strength (kg/cm²)	Shell Thickness (µm)	Albumen Height (mm)	Albumen Index (%)	Yolk Index (%)	Haugh Unit (%)	Yolk Cholesterol (mg/g yolk)
0		76.60	2.61	385.5	7.31	9.18	42.08	83.90	16.45ᵃ
1		77.33	2.66	388.2	7.33	9.34	41.66	83.86	15.02ᵇ
	0	77.20	2.64	387.6	7.39	9.37	41.58	84.08	15.40
	0.5	76.73	2.64	386.1	7.25	9.15	42.16	83.68	16.10
0	0	76.85	2.60	385.1	7.37	9.25	41.89	83.90	16.69x
1	0	77.55	2.68	390.1	7.40	9.48	41.27	84.26	14.05y
0	0.5	76.34	2.62	386.0	7.24	9.11	42.27	83.89	16.22x
1	0.5	77.12	2.65	386.3	7.26	9.20	42.05	83.46	16.00x
SEM		0.38	0.07	3.90	0.08	0.14	0.29	0.48	0.52
Two way ANOVA (P-values)									
Inactive yeast		0.064	0.439	0.502	0.802	0.252	0.164	0.948	0.013
Live yeast		0.224	0.949	0.712	0.106	0.143	0.058	0.405	0.173
Inactive yeast X Live yeast		0.922	0.650	0.561	0.967	0.614	0.498	0.417	0.032

a-b; x-y Means results within columns with different letters are significantly different (P<0.05); n = 6

Table 4. *The effects of dietary supplementation of inactive yeast and live yeast on anti-SRBC titers and blood serum parameters in laying hens*

Tablo 4. *Karma yemlere inaktif maya ve canlı maya ilavesinin yumurtacı tavuklarda SRBC'ye karşı antikor düzeyi ve kan serum parametreleri üzerine etkileri*

Inactive Yeast (g/kg)	Live Yeast (g/kg)	Anti SRBC Titer (log_2)	Total Protein (g/l)	Albumin (g/l)	Uric Acid (mg/l)	Cholesterol (g/l)	Triglyceride (g/l)	ALT (U/l)	AST (U/l)	ALP (U/l)
0		5.85	58.4	26.0	49.9	1.59	16.02	16.04	160.0	140.3
1		6.42	58.9	25.0	48.3	1.48	14.98	16.26	165.0	143.4
	0	6.38	59.5	25.5	50.0	1.51	15.15	16.22	168.0	134.2
	0.5	5.89	57.8	25.4	48.1	1.56	15.86	16.08	157.0	149.5
0	0	5.83	58.9	25.8	50.0	1.58	16.02	16.08	159.6	138.1
1	0	6.93	60.1	25.2	50.1	1.45	14.27	16.36	176.4	130.2
0	0.5	5.86	57.9	26.1	49.8	1.60	16.02	16.00	160.5	142.6
1	0.5	5.92	57.7	24.8	46.4	1.52	15.69	16.17	153.6	156.5
SEM		0.30	1.70	1.00	2.90	0.05	0.68	0.65	7.20	7.00
Two way ANOVA (P-values)										
Inactive yeast		0.065	0.770	0.338	0.580	0.051	0.142	0.737	0.502	0.674
Live yeast		0.110	0.330	0.969	0.512	0.383	0.309	0.833	0.144	0.041
Inactive yeast X Live yeast		0.093	0.672	0.748	0.558	0.636	0.306	0.933	0.115	0.135

that egg weight [9,27] and feed conversion ratio [8,10] were not affected by live yeast supplementation. However Hassanein and Soliman [9] reported that feed conversion ratio was better when live yeast was added at 4 and 8 g/kg and concluded that adding live yeast *Saccharomyces cerevisiae* can enhance the productive performance of laying hens and nutrient utilization via the inhibitory effect of yeast against pathogenic bacteria which may cause mild enteritis and malabsorption of nutrients. However in this study the level of 0.5 g/kg live yeast may be too low to show these beneficial effects.

Similarly to this study observed with inactive yeast, some researchers observed considerable improvement in egg production [5,28,29] and feed conversion ratio [5,30,31] of hens fed yeast and yeast products. This improvement may partially be attributed to the improvement of the intestinal lumen health and nutrient absorption [4,32].

In agreement with this study, some researchers found that yeast and yeast products supplementation had no effect on egg weight in laying hens [3,8,24,25,33,34]. In contrast, others reported that egg weight was increased by dietary supplementation with yeast and yeast products [4,5,26]. It was also observed that there were no interactions in feed intake, egg production, egg weight and feed conversion ratio between inactive yeast and live yeast. The differences between the results of this study and previous studies may be the age of hens, dietary nutrient composition, type and level of yeast and yeast products.

Dietary inactive yeast or live yeast had no significant effect on the internal and external egg quality characteristics and no interactions were seen between inactive yeast and live yeast in these egg characteristics. In agreement with the present study some researchers [4,5,7] had not observed any effect on egg quality characteristics. However Chumpawadee et al.[10] reported that cassava yeast as probiotic source had positive effect on shell thickness of laying hens. Hassanein and Soliman [9] also observed that egg shell thickness was improved due to feeding various yeast levels and explained that this improvement may be attributed to the enhancement of calcium absorption and retention associated with adding yeast.

Egg yolk cholesterol concentration was decreased significantly with inactive yeast supplementation (P=0.013) but was not affected by live yeast supplementation in the present study. Inactive yeast supplementation in the absence of live yeast decreased egg yolk cholesterol concentration significantly compared to other groups. Some researchers also observed that egg yolk cholesterol was reduced by yeast probiotics [35,36] and yeast and yeast products [4-7]. The reduction in yolk cholesterol could be explained by the reduced absorption, synthesis or both of cholesterol in the gastrointestinal tract [36].

Antibody titers against SRBC were not affected by the supplementation of inactive yeast and live yeast and no interactions were seen in antibody titers. However inactive yeast supplementation tended to increase antibody titers against SRBC (P=0.065). Yalçın et al.[5] observed that greater antibody titer in laying hens fed diets containing 2, 3 or 4 g/kg yeast autolysate. Mohiti-Asli [25] reported that immune response of laying hens with multistrain probiotic and yeast supplementation was greater than the control group. Prebiotics would bind to macrophage reception sites by recognizing specific sugars found in glycoproteins of the epithelial surface, triggering a cascading reaction that would activate macrophages and release cytokines, thereby activating the acquired immune response and causing the higher antibody responses against antigens [37,38]. This may be an explanation for higher antibody titers in hens fed inactive yeast as prebiotics.

Dietary supplementation of inactive yeast and live yeast did not affect blood serum levels of total protein, albumin, uric acid, cholesterol, triglyceride, ALT, AST and ALP. In addition no interactions were seen in these blood serum parameters. However dietary inactive yeast tended to reduce serum yolk cholesterol (P=0.051). Yalçın et al.[5] observed that serum cholesterol and triglyceride was reduced with the addition of 2, 3 and 4 g/kg yeast autolysate. Krasowska et al.[39] reported that baker's yeast *Saccharomyces cerevisiae* can be the best organism for reducing cholesterol in the gastrointestinal system. Similar to the present study, Yalçın et al.[5] showed that yeast autolysate supplementation had no effect in the levels of serum total protein and AST. Saoud and Daghir [40] also reported that the level of serum uric acid was not affected with dietary single cell protein. In other study, Yalçın et al.[4] observed that serum levels of total protein, triglyceride, cholesterol, AST and ALP were not affected by yeast culture supplementation.

The differences between the results of the present study and those of previous studies may be due to the heterogeneity of the experimental protocol utilized: species and age of birds, dietary nutrient composition, type and dosage of yeasts in the diets, survivability of live microorganisms in probiotic yeasts and environmental conditions.

As a result dietary inactive yeast at 1 g/kg had beneficial effects in laying performance and in low cholesterol-egg production. No adverse effects were seen on other parameters. Further researches with high doses are required to see the effects of live yeast and to determine the mechanism of actions, evaluating inactive yeast and live yeast interaction.

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REFERENCES

1. Gaggia F, Mattarelli P, Bruno B: Probiotics and prebiotics in animal feeding for safe food production. *Int J Food Microbiol*, 141 (Suppl 1): S15-S28, 2010. DOI: 10.1016/j.ijfoodmicro.2010.02.031

2. Hosseini SA, Lotfollahian H, Kamyab A, Mahdavi A: Study on the effect of yeast (*Saccharomyces cerevisiae SC47*) utilization on the commercial layer hen's performance. *Pak J Biol Sci*, 9, 2346-2349, 2006. DOI: 10.3923/pjbs.2006.2346.2349

3. Sacakli P, Ergun A, Koksal BH, Ozsoy B, Cantekin Z: Effects of inactivated brewer's yeast *(Saccharomyces cerevisiae)* on egg production, serum antibody titres and cholesterol levels in laying hens. *Vet Med Zoot*, 61, 53-60, 2013.

4. **Yalçın S, Özsoy B, Erol H, Yalçın S:** Yeast culture supplementation to laying hen diets containing soybean meal or sunflower seed meal and its effect on performance, egg quality traits and blood chemistry. *J Appl Poult Res*, 17, 229-236, 2008. DOI: 10.3382/japr.2007-00064

5. Yalçın S, Yalçın S, Çakın K, Eltan Ö, Dağaşan L: Effects of dietary yeast autolysate *(Saccharomyces cerevisiae)* on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. *J Sci Food Agric*, 90, 1695-1701, 2010. DOI: 10.1002/jsfa.4004

6. Yalçın S, Yalçın S, Onbaşılar İ, Eser H, Şahin A: Effects of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens. *Ankara Üniv Vet Fak Derg*, 61, 289-294, 2014.

7. Yalçın S, Yalçın S, Uzunoğlu K, Duyum HM, Eltan Ö: Effects of dietary yeast autolysate *(Saccharomyces cerevisiae)* and black cumin seed *(Nigella sativa* L.) on performance, egg traits, some blood characteristics and antibody production of laying hens. *Livest Sci*, 145, 13-20, 2012. DOI: 10.1016/j.livsci.2011.12.013

8. Yousefi M, Karkoodi K: Effect of probiotic Thepax[R] and *Saccharomyces cerevisiae* supplementation on performance and egg quality of laying hens. *Int J Poult Sci*, 6, 52-54, 2007.

9. Hassanein SM, Soliman NK: Effect of probiotic *(Saccharomyces cerevisiae)* adding to diets on intestinal microflora and performance of Hy-Line layers hens. *J Am Sci*, 6, 159-169, 2010.

10. Chumpawadee S, Chantiratikul A, Sataweesuk S: Effect of dietary inclusion of cassava yeast as probiotic source on egg production and egg quality of laying hens. *Int J Poult Sci*, 8, 195-199, 2009. DOI: 10.3923/ijps.2009.195.199

11. Dizaji SB, Pirmohammadi R: Effect of *Saccharomyces cerevisiae* and Bioplus 2B on performance in laying hens. *Int J Agric Biol,* 11, 495-497, 2009.

12. Ayaşan T, Özcan BD, Baylan M, Canoğulları S: The effects of dietary inclusion of probiotic protexin on egg yield parameters of Japanese quails *(Coturnix coturnix Japonica)*. *Int J Poultry Sci*, 5, 776-779, 2006.

13. Hy-line International: Management Guide - Hy-line Brown Commercial Layers. www.hyline.com, 2014.

14. European Union Directive: Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. *Official J Europ Union,* 20.10.2010. 276/33-79, 2010.

15. AOAC: Official Methods of Analysis of AOAC International. 17th ed., Association of Official Analytical Chemists, AOAC International, Gaithersburg, MD, 2000.

16. ADAS: The Analysis of Agricultural Materials. Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service. 2nd ed., Her Majesty's Stationery Office, London, 1981.

17. Farese G, Schmidt JL, Mager M: An automated method for the determination of serum calcium with glyoxal bis (2-hydroxyanil). *Clin Chem,* 13, 515-520, 1967.

18. Carpenter KJ, Clegg KM: The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *J Sci Food Agric,* 7, 45-51, 1956. DOI: 10.1002/jsfa.2740070109

19. Waldroup PW, Ndide LI, Hellwig HM, Hebert JA, Berrio L: Influence of probucol (4,4'-isopropylidine dithio)-bis(2,6-di-t-butyl-phenol) on egg yolk cholesterol content and performance of laying hens. *Poult Sci*, 65, 1949-1954, 1986.

20. TECO: *Cholesterol (Liquid) reagent*. C507. Teco Diagnostics. Anaheim, USA, 2001.

21. Onbaşılar EE, Aksoy T: Stress parametrs and immune response of layers under different cage floor and density conditions. *Livest Prod Sci,* 95, 255-263, 2005. DOİ: 10.1016/j.livprodsci.2005.01.006

22. Vitros: Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, USA, 2010.

23. Dawson B, Trapp RG: Basic and Clinical Biostatistics. 3rd ed., Lange Medical Books/McGraw-Hill Medical Publishing Division, New York, 2001.

24. Abubakar A, Tukur HM, Sekoni AA, Hassan WA: Performance and egg quality characteristics of laying birds fed diets containing rice bran with and without yeast supplementation. *Asian J Anim Sci,* 1, 1-9, 2007. DOI: 10.3923/ajas.2007.1.9

25. Mohiti Asli M, Hosseini SA, Lotfollahian H, Shariatmadan F: Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. *Int J Poult Sci*, 6, 895-900, 2007.

26. Ayanwale BA, Kpe M, Ayanwale VA: The effect of supplementing *Saccharomyces cerevisiae* in the diets on egg laying and egg quality characteristics of pullets. *Int J Poult Sci*, 5, 759-763, 2006.

27. Nursoy H, Kaplan O, Oğuz MN, Yılmaz O: Effects of varying levels of live yeast culture on yield and some parameters in laying hen diets. *Indian Vet J*, 81, 59-62, 2004.

28. Berry WD, Lui P: Egg production, egg shell quality and bone parameters in broiler breeder hens receiving Bio Mos and Eggshell 49. *Poult Sci,* 79 (Suppl 1): 124 (Abstr), 2000.

29. Stanley VG, Brown C, Sefton T: Single and combined effects of dietary protease and mannanoligosaccharide on the performance of laying hens. *Poult Sci,* 79 (Suppl 1): 62 (Abstr), 2000.

30. Kumprecht I, Zobac P, Sikse V, Sefton AE, Spring P: Effects of dietary mannanoligosaccharide level on performance and nutrient utilization of broilers. *Poult Sci,* 76 (Suppl 1): 132 (Abstr), 1997.

31. Savage TF, Zakrzewska EI, Andreasen JR: The effects of feeding mannanoligosaccharide supplemented diets to poults on performance and morphology of small intestine. *Poult Sci,* 76 (Suppl 1): 139 (Abstr), 1997.

32. Santin E, Malorka A, Macari M, Grecco M, Sanchez JC, Okada TM, Myasaka AM: Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *J Appl Poult Res*, 10, 236-244, 2001. DOI: 10.1093/japr/10.3.236

33. Day EJ, Dilworth BC, Omar S: Effect of varying levels of phosphorus and live yeast culture in caged layer diets. *Poult Sci*, 66, 1402-1410, 1987. DOI: 10.3382/ps.0661402

34. McKillop N, MacLsaac J, Rathgeber B: Feeding White Leghorn hens yeast beta-glucans to influence egg quality. *Poult Sci*, 85 (Suppl): 101 (Abstr), 2006.

35. Mahdawi AH, Rahmani HR, Pourreza J: Effect of probiotic supplements on egg quality and laying hen performance. *Int J Poult Sci*, 4, 488-492, 2005.

36. Mohan B, Kadirvel M, Bhaskaran M, Natarajan A: Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br Poult Sci*, 36, 799-803, 1995. DOI:10.1080/00071669508417824

37. Newman K: Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and immune system. Lyons TP, Jacques KA (Ed): Biotechnology in the Feed Industry: *Proceedings of Alltech's Tenth Annual Symposium.* pp.165 174, Nottingham Univ Press, Nottingham, UK, 1994.

38. Silva VK, Silva JDT, Torres KAA, Faria Filho DE, Hada FH, Moraes VMB: Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. *J Appl Poult Res,* 18, 530-540, 2009. DOI: 10.3382/japr.2009-00004

39. Krasowska A, Kubik A, Prescha A, Lukaszewicz M: Assimilation of omega 3 and omega 6 fatty acids and removing of cholesterol from environment by *Saccharomyces cerevisiae* and *Saccharomyces boulardii* strains. *J Biotechnology*, 131S, S63-S64 (Abstr), 2007. DOI: 10.1016/j.jbiotec.2007.07.109

40. Saoud NB, Daghir NJ: Blood constituents of yeast fed chicks. *Poult Sci*, 59, 1807-1811, 1980. DOI: 10.3382/ps.0591807