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Effects of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens

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Summary: The objective of this study was to determine the effects of dietary yeast cell wall (YCW) on performance, egg quality, some blood parameters and humoral immune response of laying hens during 26 wks period. For this purpose a total of 225 Hyline Brown laying hens, 39 wks of age, were allocated to one control group and four treatment groups. Basal diet was supplemented with YCW derived from bakers yeast *Saccharomyces cerevisiae* (InteMOS) at the level of 1, 2, 3 and 4 g/kg in the diets of the first, second, third and fourth treatment groups, respectively. Dietary treatments did not significantly affect body weight, feed intake, egg production, egg weight, feed conversion, and egg internal and external quality characteristics. YCW supplementation at the level of 1 and 2 g/kg decreased egg yolk cholesterol level as mg per g yolk ($P < 0.05$). Blood serum levels of cholesterol and triglyceride were decreased with the dietary inclusion of YCW at the level of 2, 3 and 4 g/kg ($P < 0.01$). Dietary YCW supplementation increased antibody titres to SRBC ($P < 0.01$). As a result dietary YCW at the level of 1 and 2 g/kg had beneficial effects in the production of low cholesterol eggs and improvement in humoral immunity response.

Key words: Egg quality, immune response, laying hen, performance, yeast cell wall.

Yumurta tavuğu rasyonlarına maya hücre duvarı ilavesinin performans, yumurta kalitesi ve humoral immun yanıt üzerine etkileri

Özet: Bu araştırma, yumurta tavuğu rasyonlarına maya hücre duvarı ilavesinin 26 hafta süreyle performans, yumurta kalitesi, bazı kan parametreleri ve humoral immun yanıt üzerine etkilerini belirlemek amacıyla yapılmıştır. Bu amaçla 39 haftalık 225 adet Hyline kahverengi yumurta tavuğu 1 kontrol ve 4 deneme grubuna ayrılmıştır. Kontrol grubu rasyonuna ekmek mayası *Saccharomyces cerevisiae* (InteMOS)'den elde edilen maya hücre duvarı 1, 2, 3 ve 4 g/kg düzeylerinde ilave edilerek sırasıyla birinci, ikinci, üçüncü ve dördüncü deneme grupları rasyonları oluşturulmuştur. Rasyonlara maya hücre duvarı ilavesi canlı ağırlığı, yem tüketimini, yumurta verimini, yumurta ağırlığını, yemden yararlanmayı ve yumurta iç kalite ile dış kalite özelliklerini etkilememiştir. Maya hücre duvarının 1 ve 2 g/kg düzeylerinde ilavesi ile yumurta kolesterol düzeyi (mg/g yumurta sarısı) azalmıştır ($P<0.05$). Kan serumu kolesterol ve trigliserit düzeyleri rasyona 2, 3 ve 4 g/kg maya hücre duvarı ilavesi ile azalmıştır ($P<0.01$). Rasyona maya hücre duvarı ilavesi SRBC'ye karşı antikor titresini artırmıştır ($P<0.01$). Sonuç olarak rasyona 1 ve 2 g/kg düzeyinde maya hücre duvarı ilavesi düşük kolesterollü yumurta üretiminde ve humoral immun yanıtın gelişmesinde yararlı olacağı kanısına varılmıştır.

Anahtar sözcükler: İmmun yanıt, maya hücre duvarı, performans, yumurta kalitesi, yumurta tavuğu.

Introduction

Yeasts and yeast products serve as alternatives to antibiotics for growth promotion and disease resistance in poultry. β-glucans and mannanoligosaccharides are major components of the YCW. The composition of the YCW depends on the cultivation conditions (14). The mannan oligosaccharides (MOS), derived from the outer cell wall of yeast, shift gastrointestinal microflora balance toward beneficial organisms (27). (1→3), (1→6)-β-D-glucan from *Saccharomyces cerevisiae* is a well-known immunomodulator (17).

There are some reports about the usage of various yeast and yeast products such as inactive dried yeast, yeast culture, whey yeast, selenium yeast, chromium yeast and yeast autolysate in the diets of laying hens on performance (13, 31, 32, 34). Yalçın et al. (2010) concluded that dietary yeast autolysate at the levels of 2, 3 and 4 g/kg had beneficial effects on performance, egg cholesterol content and humoral immune response. In some studies YCW supplementation improved the gastrointestinal microflora balance, performance and antibody responses in poultry (8, 27). Gürbüz et al.

(2011) reported that 0.5% YCW supplementation increased egg production but had no effect on egg weight and feed conversion in laying hens. Therefore, the present study was aimed to examine the effects of different levels of YCW derived from bakers yeast, *Saccharomyces cerevisiae*, as a feed additive on performance, egg traits, egg cholesterol concentration, some blood parameters and humoral immune response of laying hens.

Material and Methods

Material: A total of 225 Hyline Brown laying hens aged 39 wk were used in this study. Hens were randomly allocated into one control group and four treatment groups each containing 45 hens. Each group was divided into five replicates as subgroups, comprising nine hens each. They were housed in cages (30cm x 44cm x 44cm) 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 26 wk experimental period. The ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was supplemented with YCW derived from bakers yeast, *Saccharomyces cerevisiae* (InteMOS, Integro Food and Feed Manufacturing Company, İstanbul, Turkey) at the level of 1, 2, 3 and 4 g/kg for the diets of the first, second, third and fourth treatment groups, respectively. The chemical composition of the YCW is presented in Table 2.

Traits measured: Nutrient composition of YCW and basal diet were determined according to the AOAC (3). The samples were ashed in a muffle furnace prior to the analysis of calcium (11) and total phosphorus (2). Other minerals were determined with ICP-MS system (Agillent 7500 ce model, serial no: JP51201902, Yokogawa Analytical Systems, Yamanashi-Ken, Japan). Metabolizable energy levels of samples were estimated using the equation of Carpenter and Clegg (7). Free and total amino acids of YCW were determined with modified OPA derivatization using the HPLC system of Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) (Heems et al., 1998).

Table 1: Ingredients and chemical composition of the basal diet.
Tablo 1. Bazal rasyonun içeriği ve kimyasal bileşimi.

Ingredients (g/kg)		Chemical composition (Analyzed)	
Corn	570.3	Metabolizable energy[b] (kcal/kg)	2810
Soybean meal	178.0	Crude protein (g/kg)	176.2
Full fat soya	130	Calcium (g/kg)	41.0
Limestone	96	Total phosphorus (g/kg)	7.0
Dicalcium phosphate	18		
Salt	2.7		
DL-Methionine	2.0		
Lysine	0.5		
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 µg vitamin B_{12}, 25 mg niacin, 8 mg calcium-D-panthotenate, 1 mg folic acid, 50 mg vitamin C, 50 µg 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 [8].

Table 2: Chemical composition of yeast cell wall.
Tablo 2. Maya hücre duvarının kimyasal bileşimi.

			Free amino acids (g/kg)	Total amino acids (g/kg)
Dry matter (g/kg)	935.5	Aspartic acid	0.05	16.10
ME (MJ/kg)	5.17	Glutamic acid	1.54	24.40
Crude protein (g/kg)	227.2	Serine	0.07	7.04
Ether extract (g/kg)	37.4	Histidine	0.02	4.76
Crude fibre (g/kg)	4.3	Glycine	0.15	11.18
Crude ash (g/kg)	64.0	Threonine	0.02	5.31
		Arginine	0.07	6.22
		Alanine	0.67	13.01
Minerals (mg/kg)		Tyrosine	0.06	7.77
Calcium	7390	Valine	0.05	12.32
Phosphorus	5906	Methionine	0.01	3.96
Magnesium	1230	Phenylalanine	0.06	12.62
Sodium	15750	Isoleucine	0.02	11.72
Potassium	5829	Ornithine	<0.03	5.76
Zinc	130	Leucine	0.03	19.04
Manganese	36	Lysine	0.02	15.69
		Hyroxyproline	<0.3	8.05
		Proline	1.00	14.91

Hens were weighed individually at the beginning and at the end of the experiment. Mortality was recorded as it occurred. 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. The fresh excreta samples from each replicate in each group were collected using a plastic tray at the first and the second day of the 24^{th} week of the experiment. Care was taken to collect fresh excreta which had no contact with drinking water. All excreta samples were dried in an air-forced oven at 60^{o}C until reaching constant weight, and then, moisture of samples was determined according to AOAC (3).

To determine the egg internal and shell quality characteristics, 100 eggs laid at 0900 to 1200 h were collected randomly from each group (20 eggs from each replicate in total) during four consecutive days of last week. Each egg was weighed and their shape index was measured with a special instrument (B.V. Apparatenfabreik Van Doorn, No: 75 135/2, De Bilt, Holland). Egg shell breaking strength was measured by using an egg breaking tester (static compression device, Dr.-Ing. Georg Wazau Mess - und Prüfsysteme GmbH, Berlin, Germany). The egg content was broken onto a glass-topped table. Egg shell thickness was measured in three different parts (upper and lower ends and middle) using a micrometer (Mitutoya, No. 1044N, 0.01-5 mm; Kawasaki, Japan). Then the height of the albumen and the yolk was measured with a tripod micrometer (Mitutoya, No. 2050-08, 0.01-20 mm; Kawasaki, Japan). The length and width of the albumen and the diameter of the yolk were measured using a digital caliper. By using these values, yolk index [(yolk height / yolk diameter) x 100], albumen index [(albumen height / average of albumen length and albumen width) x 100] and Haugh units [100 x log(H + 7.57 - $1.7W^{0.37}$), where H is albumen height and W is egg weight] were calculated (6). Egg internal and external quality analysis were completed within 24 h of the eggs being collected. Egg quality evaluation was performed for individual eggs, as it was done in relation to egg weight.

At the end of the experiment, 20 eggs per each group (4 eggs from each replicate) were randomly chosen to determine yolk cholesterol. Eggs were boiled for 5 min. They were allowed to cool and then broken and their constituent parts were separated and weighed. The shells were weighed after being air-dried for 24 h. The percentage values of shell weight, yolk weight and albumen weight were calculated. Egg yolk was blended with isopropyl alcohol with a volume of 10 ml per g of yolk (30). Cholesterol content of this extract was determined according to the enzymatic method of TECO (29). Yolk cholesterol was calculated and expressed as mg per g yolk.

At the 23rd wk of the experiment, 15 hens were randomly selected from each group (3 from each replicate) and 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 microhemagglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution (22).

Blood samples were collected from vena brachialis under the wing from 15 fed hens randomly chosen from each group (three from each replicate) at the end of the experiment and centrifuged at 3000 x g for 10 min. Serum was collected and stored at -20^{o}C for determination of total protein, uric acid, triglyceride, cholesterol and levels of aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) by Vitros 350 autoanalyser (New York, USA; Product code 680-2153) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, USA).

Statistical analyses: Statistical analyses were done using SPSS programme (SPSS Inc., Chicago, IL, USA). The normality of data distribution was checked using the Kolmogorov-Smirnov test. One-way ANOVA was performed to examine the differences among groups. The significance of mean differences between groups were tested by Tukey. Egg internal and external quality characteristics were analyzed after adjusting egg weight and values were given as estimated marginal means and standard error of mean. Level of significance was taken as $P < 0.05$ (9).

Results

YCW contains high amount of glutamic acid (24.40 g/kg), leucine (19.04 g/kg), aspartic acid (16.10 g/kg) and lysine (15.69 g/kg) but low amount of methionine and threonine as shown in Table 2. The inclusion of YCW in the diet of laying hens did not significantly affect body weight, feed intake, hen day egg production, egg weight, feed conversion ratio and excreta moisture (Table 3). During the experimental period one hen died in each of the groups fed with diets containing YCW at the level of 0 and 4 g/kg. YCW supplementation had no significant effect on the mean values of egg shape index, egg breaking strength, egg shell thickness, yolk index, albumen height, albumen index and Haugh unit and the percentages of egg parts (Table 4). Egg yolk cholesterol content was lowered in all of the YCW-supplemented groups as compared to the control group ($P<0.05$). Dietary YCW supplementation increased ($P<0.01$)

Table 3: The effects of dietary supplementation of yeast cell wall on performance and excreta moisture of laying hens.
Tablo 3. Rasyona maya hücre duvarı ilavesinin yumurta tavuklarında performans ve dışkı nemi üzerine etkileri.

	Yeast cell wall (g/kg)					SEM	P
	0	1	2	3	4		
Initial body weight (g)	1858	1879	1887	1803	1868	13	0.348
Final body weight (g)	1841	1842	1825	1805	1843	12	0.863
Feed intake (g/day per hen)	107.3	107.8	107.8	107.7	107.0	0.11	0.062
Hen-day egg production (%)	84.7	86.1	86.2	86.9	85.7	0.32	0.300
Egg weight (g)	63.6	64.0	63.9	64.0	63.8	0.15	0.917
Feed efficiency (kg feed per kg egg)	1.99	1.96	1.96	1.94	1.96	0.01	0.199
Excreta moisture (g/kg)	751.7	743.1	744.5	744.5	733.9	2.8	0.432

No significant differences among groups.

Table 4: The effects of dietary supplementation of yeast cell wall on egg traits of laying hen.
Tablo 4: Rasyona maya hücre duvarı ilavesinin yumurta tavuklarında yumurta kalitesi üzerine etkisi.

	Yeast cell wall (g/kg)					SEM	P
	0	1	2	3	4		
Shape index (%)	78.42	78.03	77.54	77.67	77.05	0.32	0.068
Breaking strength (kg/cm^2)	2.71	2.74	2.76	2.78	2.76	0.03	0.554
Shell thickness, (μm)	356.9	357.9	357.5	358.2	357.6	0.33	0.999
Albumen height, (mm)	6.90	6.95	6.89	6.90	6.87	0.09	0.971
Albumen index (%)	8.94	9.19	8.98	9.11	9.01	0.16	0.773
Yolk index (%)	39.75	39.77	39.82	39.88	39.76	0.37	0.999
Haugh unit (%)	81.95	82.26	81.91	81.97	81.73	0.59	0.979
Albumen percentage (%)	59.83	59.76	59.79	60.73	59.23	0.19	0.166
Yolk percentage (%)	28.16	27.95	27.85	27.45	28.36	0.17	0.549
Shell percentage (%)	12.01	12.29	12.36	11.82	12.41	0.11	0.384
Yolk cholesterol (mg/g yolk)	16.78a	14.58b	14.54b	14.63ab	15.06ab	0.28	0.028

[a,b] Means within a row followed by the different superscripts differ significantly (P < 0.05).

Table 5: The effects of dietary supplementation of yeast cell wall on anti-SRBC titer, and some blood serum parameters in laying hens.
Tablo 5. Rasyona maya hücre duvarı ilavesinin anti-SRBC titresi ve bazı kan parametreleri üzerine etkileri.

	Yeast cell wall (g/kg)					SEM	P
	0	1	2	3	4		
Anti SRBC titer (log_2)	5.33 b	6.20a	6.40a	6.40a	6.53a	0.12	0.002
Total protein (g/l)	46.2	45.0	48.6	46.9	47.9	0.5	0.261
Uric acid (mg/l)	39.4	40.7	40.3	40.2	39.7	0.9	0.995
Cholesterol (g/l)	1.57a	1.59a	1.35b	1.34b	1.35b	0.03	0.004
Triglyceride (g/l)	17.98a	15.28b	14.96b	14.80b	14.89b	0.35	0.006
AST (U/l)	155.8	156.3	156.9	151.9	154.8	2.8	0.987
ALP (U/l)	247.8	265.0	273.5	287.9	275.9	6.5	0.406
ALT (U/l)	15.07	15.57	15.20	15.93	14.87	0.46	0.963

[a,b] Means within a row followed by the different superscripts differ significantly (P < 0.05).

antibody titres to SRBC (Table 5). There were no significant differences in the serum levels of total protein, uric acid, AST, ALP and ALT. Dietary supplementation with YCW at 2, 3 and 4 g/kg reduces ($P < 0.01$) the levels of serum cholesterol and triglyceride (Table 5).

Discussion and Conclusion

Dietary supplementation of YCW did not significantly affect body weight, feed intake, hen day egg production, egg weight and feed conversion of laying hens (Table 3). Similar results have also been obtained by some researchers (1, 20, 23). However some researchers (5, 12, 28) have reported considerable improvement in egg production in poultry fed MOS and YCW. The improvement in egg production in layers and breeders might be explained that the MOS reduces the pathogenic bacteria load in the intestine, then the nutrients in the diets are efficiently diverted toward production in poultry fed MOS, which might improve egg production in layers and breeders (26, 27). Similar to the present experiment some researchers have found that

yeast and yeast products supplementation had no effect on egg weight in laying hens (1, 10, 12, 18, 20, 34). In contrast, others reported that egg weight increased by the dietary supplementation of yeast and yeast products (4, 17, 31, 32). Some researchers (16, 25) observed that feed conversion improved when broilers and poults were fed diets supplemented with MOS. Yalçın et al. (32) reported that feed efficiency was improved with yeast autolysate supplementation at the level of 2, 3 and 4 g/kg ($P < 0.05$). In the present study mortality was not treatment related. These data are consistent with the findings of researchers (1, 12, 23, 31, 34) involving laying hens fed diets supplemented with yeast and yeast products.

YCW supplementation had no significant effect on the mean values of egg shape index, egg breaking strength, egg shell thickness, yolk index, albumen height, albumen index and Haugh unit and the percentages of egg parts (Table 4). Similarly yeast culture (31) and yeast autolysate (32) supplementation did not significantly affect interior and exterior egg quality characteristics. McKillop et al. (18) also reported that albumen height was not affected by the yeast beta-glucan supplementation at the level of 25, 50 and 250 g/tonne. However Ayanwale et al. (4) observed that egg shell weight and yolk weight were higher in laying hens fed diets having 7.5 g/kg dried yeast. Özek (23) also found that dietary MOS supplementation significantly modified albumen height and Haugh unit.

A significant reduction ($P < 0.05$) in egg yolk cholesterol content as mg per g yolk was observed in the groups fed diets supplemented 1 and 2 g/kg (Table 4). Similarly some researchers (31-34) observed that egg yolk cholesterol values were decreased by dietary supplementation of yeast or yeast products. This reduction in yolk cholesterol might be explained by the reduced absorption, synthesis or both of cholesterol in the gastrointestinal tract (19).

There was a significantly higher antibody titre ($P<0.05$) in the groups fed diets supplemented with YCW as compared to the control group (Table 5). This higher antibody titre in laying hens supplemented with yeast cell wall could be explained by the beneficial effects of supplementation in maintaining physiological balance of immunopotent cells and therefore a healthy environment for the immune system will be provided. Similarly, some researchers (8, 26) reported that higher antibody responses observed in the broiler breeders fed MOS. However Özek (23) supplementing diets with MOS had no significant advantageous effect on serum antibody titres against ND, IB and IBD viruses in the summer season in laying hens.

Dietary supplementation with YCW at 2, 3 and 4 g/kg reduces the levels of serum cholesterol and triglyceride ($P < 0.01$) (Table 5). However there were no significant differences in the serum levels of total protein, uric acid, AST, ALP and ALT. Similar results about serum cholesterol, triglyceride, protein, uric acid and the activities of ALT (32). Krasowska et al. (15) reported that baker's yeast *Saccharomyces cerevisiae* seem to be perfect organism for reducing cholesterol in the gastrointestinal tract. Nicolasi et al. (21) also reported that the yeast derived β-glucan significantly lowered total cholesterol concentrations in hypercholesterolemic men. Yalçın et al. (31) reported that serum levels of total protein, triglyceride, cholesterol, AST and ALP were not affected by the addition of yeast culture. Saoud and Daghir (24) reported that single cell protein had no effect on serum uric acid in broilers. Özek (23) also observed that MOS supplementation had not significantly modified serum triglyceride and total cholesterol concentrations.

The differences between the results of the present study and those of previous studies may be the species and age of the birds, dietary nutrient composition, type, dosage and composition of yeast cell wall in the diets and environmental conditions.

As a result, dietary supplementation of YCW at the level of 1 and 2 g/kg in laying hens had beneficial effects on egg cholesterol content and humoral immunity. Serum cholesterol and triglyceride levels were reduced by YCW supplementation at the level of 2, 3 and 4 g/kg. No adverse effects were seen on the other parameters. The supplementation of YCW derived from bakers yeast (*Saccharomyces cerevisiae*) has potential commercial applications for improvement in the production of low cholesterol eggs and humoral immunity.

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