



The role of yeast culture (*Saccharomyces cerevisiae*) on performance, egg yolk fatty acid composition, and fecal microflora of laying hens

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ABSTRACT - This study investigated the effects of different levels (0.05, 0.1, and 0.2%) of yeast culture supplementation on body weight, feed intake, feed conversion ratio, egg production, egg weight, egg quality traits, egg yolk fatty acid composition, and microbiological flora in feces. A total of 240 laying hens at 18-19 weeks of age were divided into four groups and fed a basal diet containing 2750 kcal/kg metabolizable energy and 16% crude protein for 16 weeks. The basal diet was supplemented with 0.05, 0.1, and 0.2% commercial yeast culture product obtained from *Saccharomyces cerevisiae*. The different levels of yeast culture supplementation to the diets did not statistically affect body weight change among the treatments. However, feed intake was lowest in the group fed 0.2% of yeast culture. The highest egg weights were obtained from the groups fed 0.1 and 0.2% yeast culture, when compared with control group. Regarding fatty acid composition, linolenic acid (C18:2 n6) was lowest in the group fed 0.2% yeast culture. However, yeast culture supplementation to the diet did not alter the microbial flora. Yeast culture (*S. cerevisiae*) supplementation to the diet of laying hens is beneficial for increasing feed intake and egg weight of laying hens without affecting the microbial flora in their digestive system.

Key Words: laying hen, fatty acid, microbiological flora, yeast culture

Introduction

Feed additives, which are defined as substances, have long been added to animal diets to increase the utilization of feeds as well as the quantity and quality of animal products. Yeast cultures are complex fermented products containing metabolic agents produced by yeast during fermentation. Live yeast cultures contain digestive enzymes such as amylase, maltase, sucrose, lactate dehydrogenase, proteinase, polypeptides, dipeptidase, deaminase, transaminase, lipase, phospholipase, phosphatase, and phytase and provide higher digestibility (Stanley et al., 1993). *Saccharomyces cerevisiae*, which produces these properties, has been proven to have beneficial effects on poultry production, e.g., in reproduction, growth rate, egg production, and feed efficiency (Dawson, 1993). In addition, supplementation of yeast, yeast extracts, and yeast cultures to feed has

provided economic and environmental benefits in poultry diets for the past 40 years (Stone, 2007). *Saccharomyces cerevisiae* is known to increase the biological value of nitrogenous compounds in the digestive tract and to reduce stress-producing factors in animals. Yeast culture also helps digestion by increasing the availability of nutrients (Stanley et al., 1993).

The positive effects of supplementation of yeast culture, which might also be called probiotics on the performance values of animals, have been attributed to many factors including (but not limited to) microbial flora, dose added to the feed, environmental conditions, and physical condition of the animal (Ceylan and Çiftçi, 2003). It has been shown that probiotic microorganisms reach the villi of the intestinal lumen earlier than pathogenic bacteria, thus preventing these pathogens from becoming trapped in the digestive tract (Hassanein and Soliman, 2010).

In other words, probiotic microorganisms compete with pathogenic microorganisms for binding to receptors (competitor exclusion). The positive effect may also be due to the strengthening of the natural defense system and the biological regulation of intestinal microflora of the host, as well as to the direct effect of the probiotic on health or the

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nutritional form of the probiotic (Shareef and Al-Dabbagh, 2009; Hassanein and Soliman, 2010).

This study aimed to investigate the effects of the different levels (0.05, 0.1, and 0.2%) of yeast culture supplementation on feed intake (FI), feed conversion ratio (FCR), egg production, egg quality traits, egg yolk fatty acid composition, and microbiological flora in the feces of laying hens.

Material and Methods

A total of 240 laying hens at 18-19 weeks of age were divided into four groups (control and three trial groups) and fed for 16 weeks. Each group comprised 10 sub-groups, with each sub-group having six animals. The animals were fed a basal diet containing 2750 kcal/kg metabolizable energy and 16% crude protein (Table 1). The yeast culture (*S. cerevisiae*) was added to the diet at different levels (0.05, 0.1, and 0.2% body weight – BW). The BW was determined at the beginning and at the end of the study. The daily egg production of the groups was measured. The FI and FCR of animals were recorded weekly (Table 2).

In the 4th, 8th, 12th, and 16th weeks of the study, the egg internal and external quality traits were determined in 10 randomly selected eggs from each group, and measurements were repeated four times. Eggs were weighed twice a week for two successive days, and average weights were recorded (Table 3).

Egg yolk oil extraction was conducted using nine egg samples from each group as reported by AOAC (2000). Afterwards, methyl esters were formed with boron trifluoride. The fatty acid methyl esters were condensed under nitrogen gas and then analyzed by gas

chromatography-mass spectroscopy (HP 6890/5972). The Agilent HP88 100 × 250 × 250 µm column was used in the analysis. The column starting temperature was 120 °C, the final temperature was 230 °C, and the injector and detector temperature was 250 °C. The injection speed was set to 50:1. Helium was used as a carrier gas.

Fecal samples, which were collected from each group, were placed into sterile plastic tubes in the last week of the experiment. Stool samples were diluted as 10:1 in physiological saline (FTS) containing 0.9% NaCl. Subsequently, the samples were diluted in a ten-fold (log10) dilution series by using six tubes. Inoculation began from the lowest dilution rate. Plate count agar was used for general live (total mesophilic aerobic microorganism), MacConkey agar for coliform bacteria, and Sabouraud dextrose agar for yeast. Plate count agar was incubated in aerobic conditions for 48 h at 30 °C, MacConkey agar was incubated for 24 h at 37 °C for coliform bacteria count, and Sabouraud dextrose agar was incubated at 45 °C for 72 h (Arda, 1985; Arda et al., 1997). After incubation, the media were counted and the averages were taken. The mean numbers for all dilution steps were then determined, and microorganism counts were recorded for each sample according to these results.

One-way ANOVA was used to determine the significance of the differences between the statistical calculations and mean values of the groups. The Duncan test was employed to determine the significance among the groups (Dawson and Trapp, 2001).

Results

During the experimental period, there was no side effect on the health of animals due to supplementation of yeast culture at the test concentrations added to the feed. The different levels of yeast culture supplementation to diets did not affect BW. The lowest average FI (101.96 g/day) was obtained from the group fed 0.2% yeast culture supplementation, when compared with control group (106.43 g/day) (Table 2).

The highest egg production was obtained from the groups fed 0.2 (91.54%) and 0.1% supplementation (91.93%), while control group had an average egg production of 90.30% ($P > 0.05$).

Yolk diameter was lowest in the group fed 0.1% supplementation, followed by the 0.05% supplementation, control, and 0.2% supplementation, in that order. In comparison with the control and 0.05% supplementation groups, the highest Haugh units were obtained from the 0.2%- and 0.1%-supplementation groups, respectively (Table 4).

Table 1 - Composition and nutrient level of basal diet

Item	
Ingredient (g kg ⁻¹)	
Corn	645
Soybean meal	150
Sunflower seed meal	78
Meat and bone meal	30
DL-methionine	0.5
Limestone	82
Dicalcium phosphate	10
Salt	2
Vitamin and mineral premix ¹	2.5
Calculated nutrient level	
Metabolizable energy (kcal kg ⁻¹)	2753
Crude protein (g kg ⁻¹)	160
Ca (g kg ⁻¹)	34.5
P (g kg ⁻¹)	6.2

¹ Provides per kg: 15,372.00 mg vitamin A; 6.28 mg vitamin E; 0.64 mg vitamin K3; 27.36 mg Mn; 89 mg Fe; 25 mg Zn; 8.76 mg Cu; 0.03 mg Co; 0.05 mg I; 0.91 mg Se.

In terms of fatty acid composition (Table 5), hexadecanoic (C16:0) and oleic acids (C18:1) were the main fatty acids in the egg yolk. Myristoleic (C14:1) and hexadecanoic acid (C16:0) values were higher in the 0.1 and 0.2% yeast culture-supplementation groups, when compared with control group ($P>0.05$). Polyunsaturated fatty acids (PUFA) values were numerically lower in the 0.2% yeast culture-supplementation group than in the other

groups. However, PUFA:saturated fatty acids (SFA) and C18:2 n6 values were significantly different between the control and experimental groups ($P<0.05$).

The microbiological analysis of feces (Table 6) revealed that dietary supplementation of yeast culture did not influence the total bacterial number ($P = 0.50$). The number of coliform bacteria was lowest ($1.35\pm 0.59 \log_{10} \text{cfu/g}$) in the 0.2% yeast culture-supplementation group ($P = 0.89$).

Table 2 - Effects of yeast culture on laying hen performance

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	P-value
Initial body weight (g)	1757±19.12	1740±18.20	1758±14.34	1761±8.53	0.812
Final body weight (g)	1720±17.86	1707±16.90	1750±19.36	1756±21.23	0.211
Egg production/hen/day (%)	90.30±0.60	90.93±0.52	91.54±0.53	91.35±0.43	0.35
Feed intake (g/day per hen)	106.43±1.17a	105.23±0.85a	104.82±1.05ab	101.96±1.15b	0.03
Feed conversion ratio (g feed/g egg)	2.03±0.03	1.99±0.03	1.97±0.02	1.94±0.07	0.07

Control group: basal diet; Experimental group 1: basal diet + 0.05% yeast culture; Experimental group 2: basal diet + 0.1% yeast culture; Experimental group 3: basal diet + 0.2% yeast culture.

a-b - Means within a row with different letters are different at $P<0.05$.

Table 3 - Weekly egg weights (g)

Week	Control group	Experimental group 1	Experimental group 2	Experimental group 3	P-value
1-2	56.97±0.51 n = 55	56.83±0.44 n = 55	57.38±0.51 n = 53	56.34±0.53 n = 51	0.54
3-4	57.70±0.46 n = 55	57.69±0.46 n = 58	58.49±0.54 n = 53	58.58±0.62 n = 53	0.44
5-6	58.78±0.51 n = 58	58.52±0.44 n = 60	58.79±0.56 n = 57	59.17±0.53 n = 50	0.85
7-8	58.37±0.59 n = 56	57.86±0.51 n = 58	60.19±0.65a n = 58	60.36±0.54a n = 57	0.003
9-10	57.67±0.71b n = 51	58.28±0.64ab n = 53	58.55±0.54ab n = 56	59.97±0.55a n = 58	0.052
11-12	57.97±0.62b n = 53	58.77±0.57ab n = 52	59.80±0.65a n = 56	60.44±0.53a n = 56	0.019
13-14	58.84±0.61b n = 54	59.89±0.57ab n = 55	60.96±0.53a n = 56	60.51±0.46a n = 56	0.041
15-16	58.85±0.59b n = 51	58.16±0.55b n = 54	60.47±0.66a n = 57	60.96±0.47a n = 56	0.002
All periods	58.15±0.20b n = 433	58.24±0.19b n = 445	59.35±0.21a n = 446	59.59±0.19a n = 437	<0.00

Control group: basal diet; Experimental group 1: basal diet + 0.05% yeast culture; Experimental group 2: basal diet + 0.1% yeast culture; Experimental group 3: basal diet + 0.2% yeast culture.

a-b - Means within a row with different letters are different at $P<0.05$.

Table 4 - Effects of yeast culture on egg quality traits of laying hens

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	P-value
Egg weight (g)	60.07±0.69	58.85±0.66	59.66±0.73	59.78±0.63	0.62
Shape index (%)	78.46±0.58	77.28±0.43	77.81±0.36	78.02±0.59	0.42
Shell weight (g)	5.94±0.09	5.81±0.13	6.05±0.11	6.08±0.09	0.62
Albumen weight (g)	39.72±0.62	38.79±0.61	39.18±0.67	37.62±1.12	0.29
Yolk weight (g)	14.41±0.17	14.25±0.24	14.44±0.21	16.08±1.04	0.07
Shell weight percentage (%)	9.90±0.13	9.87±0.19	10.14±0.16	10.19±0.18	0.38
Albumen weight percentage (%)	66.04±0.36	65.85±0.49	65.58±0.45	62.91±1.69	0.06
Yolk weight percentage (%)	24.06±0.35	24.28±0.44	24.28±0.41	26.90±1.67	0.08
Yolk diameter (mm)	39.23±0.31ab	38.86±0.43ab	38.08±0.41b	40.01±0.43a	0.01
Yolk height (mm)	21.49±0.28	21.31±0.28	21.25±0.26	21.48±0.35	0.92
Haugh unit	79.86±0.30b	79.71±0.31b	80.49±0.37ab	81.18±0.29a	0.005

Control group: basal diet; Experimental group 1: basal diet + 0.05% yeast culture; Experimental group 2: basal diet + 0.1% yeast culture; Experimental group 3: basal diet + 0.2% yeast culture.

a-b - Means within a row with different letters are different at $P<0.05$.

Table 5 - Effects of dietary supplementation of yeast culture on yolk fatty acids (% of total methyl esters of fatty acids) of laying hens

Fatty acid (%)	Control group	Experimental group 1	Experimental group 2	Experimental group 3	P-value
C14:0	0.31±0.015	0.31±0.016	0.32±0.022	0.30±0.020	0.96
C14:1	0.014±0.004	0.015±0.005	0.023±0.005	0.029±0.002	0.09
C:16:0	23.96±0.44	24.14±0.35	24.07±0.34	24.79±0.32	0.41
C16:1	2.97±0.15	3.07±0.16	3.15±0.12	3.27±0.11	0.48
C17:0	0.18±0.04	0.20±0.015	0.17±0.018	0.26±0.062	0.41
C17:1	0.22±0.046	0.15±0.018	0.13±0.008	0.18±0.030	0.19
C18:0	8.87±0.23	8.24±0.23	8.30±0.20	8.47±0.16	0.15
C18:1	41.07±0.69	40.72±0.42	41.00±0.58	41.98±0.82	0.55
C18:2 n6	18.09±.54ab	19.09±0.37a	18.81±0.37a	16.80±0.79b	0.03
C18:3 n6	0.51±0.044	0.43±0.043	0.48±0.039	0.45±0.028	0.56
C18:3 n3	0.88±0.040	0.97±0.035	0.94±0.048	0.84±0.058	0.23
C20:0	0.075±0.017	0.061±0.006	0.091±0.019	0.12±0.025	0.18
C20:1	0.34±0.045	0.27±0.016	0.26±0.019	0.29±0.04	0.3
C20:2 n6	0.21±0.014	0.25±0.024	0.21±0.021	0.21±0.030	0.45
C20:3 n3	0.19±0.018	0.19±0.011	0.17±0.011	0.19±0.01	0.77
C20:4 n6	1.44±0.083	1.28±0.10	1.24±0.09	1.27±0.03	0.33
C22:6 n3	0.54±0.042	0.46±0.057	0.47±0.041	0.47±0.021	0.59
C24:0	0.12±0.013	0.11±0.005	0.14±0.018	0.10±0.007	0.10
SFA	33.54±0.39	33.10±0.39	33.12±0.40	34.04±0.27	0.25
MUFA	44.61±0.64	44.22±0.46	44.56±0.66	45.73±0.89	0.44
PUFA	21.86±0.66	22.67±0.49	22.32±0.48	20.23±0.87	0.06
UFA	66.46±0.39	66.89±0.39	66.88±0.40	65.96±0.27	0.25
PUFA:SFA	0.65±0.02ab	0.69±0.02a	0.67±0.02a	0.59±0.03b	0.03
n6	20.24±0.59	21.06±0.43	20.74±0.43	18.73±0.81	0.07
n3	1.61±0.08	1.61±0.07	1.59±0.06	1.50±0.06	0.58
n6:n3	12.65±0.37	13.16±0.47	13.14±0.38	12.50±0.17	0.47
Nutritive value ¹	2.09±0.06	2.03±0.04	2.05±0.04	20.04±0.05	0.83
Atherogenic index ²	0.15±0.005	0.14±0.003	0.14±0.004	0.15±0.003	0.23
Thrombogenic index ³	0.57±0.01	0.57±0.009	0.56±0.01	0.58±0.01	0.50

Control group: basal diet; Experimental group 1: basal diet + 0.05% yeast culture; Experimental group 2: basal diet + 0.1% yeast culture; Experimental group 3: basal diet + 0.2% yeast culture.

MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated fatty acids; SFA - saturated fatty acids.

¹ (C18:0 + C18:1)/C16:0.

² (4*C14:0 + C18:0)/UFA.

³ (C14:0 + C16:0 + C18:0)/(0.5*C18:1) + (0.5*MUFA) + (0.5*n6) + (3*n3) + (n3/n6).

a-b - Means within a row with different letters are different at P<0.05.

Table 6 - Effects of dietary supplementation of yeast culture on fecal microflora (log₁₀ cfu/g) of laying hens

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	P-value
Total bacteria	6.32±0.57	6.13±0.99	4.85±0.99	4.78±0.97	0.50
Coliform bacteria	1.71±0.58	1.79±0.29	1.80±0.39	1.35±0.59	0.89
Yeast	0.15±0.043	0.36±0.15	0.23±0.09	0.29±0.05	0.46

Control group: basal diet; Experimental group 1: basal diet + 0.05% yeast culture; Experimental group 2: basal diet + 0.1% yeast culture; Experimental group 3: basal diet + 0.2% yeast culture.

Non-significant differences P>0.05.

Discussion

Studies on laying hens reported that addition of yeast culture or yeast metabolites did not affect BW (Yousefi and Karkoodi, 2007; Yalçın et al., 2012; Yalçın et al., 2014; Hassanein and Soliman, 2010). Similarly, our results also did not show any difference in the BW of laying hens. On the other hand, some studies indicated that yeast culture supplementation to broiler diets positively affected their BW (Shareef and Al-Dabbagh, 2009; Fasina and Thanissery, 2011; Fathi et al., 2012), which could be attributed to the strains of yeast and animal species, among other factors.

Although yeast culture supplementation had no statistical effect on egg production, a numerical increase was observed. Similar results were recorded for yeast or yeast metabolite supplementation to laying hen diets in many studies (Ayanvale et al., 2006; Asli et al., 2007; Yousefi and Karkoodi, 2007; Yalçın et al., 2008; Yalçın et al., 2012; Gül et al., 2013; Sacakli et al., 2013). On the other hand, Hassanein and Soliman (2010) observed an increase in egg production in laying hens by using yeast culture supplementation at much higher concentrations (0.4, 0.8, 1.2, and 1.6%).

The different levels of yeast culture supplementation to diets significantly affected FI (P<0.05). Consistent with our

findings, yeast culture supplementation positively affected FCR of broilers (Onifade and Babatunde 1996; Miazzo et al., 2005; Shareef and Al-Dabbagh, 2009) and laying hens (El-Ella et al., 1996; Katoch et al., 2003; Yalçın et al., 2012; Gül et al., 2013; Yalçın et al., 2015). Conversely, FI and FCR was not improved by yeast culture supplementation in laying hens (Nursoy et al., 2004; Asli et al., 2007; Yousefi and Karkoodi, 2007; Hassanein and Soliman, 2010; Sacakli et al., 2013), in laying quail (Önol et al., 2003), and in broiler turkeys (Özsoy and Yalçın, 2011).

The highest egg weight was observed in hens fed 0.1 and 0.2% yeast culture during the experimental period, and this difference was statistically significant ($P < 0.05$). Our findings corroborate previous studies (Ayanwale et al., 2006; Yalçın et al., 2008; Yalçın et al., 2015; Zhong et al., 2016), but disagree with others (Nursoy et al., 2004; Asli et al., 2007; Hassanein and Soliman, 2010; Gül et al., 2013; Sacakli et al., 2013).

No statistical difference was observed for shape index, shell weight, albumen weight, or yolk height. These results are consistent with those of other studies (Asli et al., 2007; Hassanein and Soliman, 2010; Yalçın et al., 2012; Yalçın et al., 2014; Yalçın et al., 2015). Yolk weight ($P < 0.07$), yolk weight percentage ($P < 0.08$), and yolk diameter ($P < 0.01$) were the highest in the group fed 0.2% yeast culture supplementation. The lowest albumen weight percentage was also found in the third trial group ($P < 0.06$). Haugh unit was calculated as 79.86, 79.71, 80.49, and 81.18 in the control and the three trial groups, respectively. The best Haugh unit was found in the third trial group ($P < 0.005$). Similarly, previous studies (Ayanwale et al., 2006; Yousefi and Karkoodi, 2007; Asli et al., 2007; Zhong et al., 2016) reported that egg yolk weight was increased by the supplementation of yeast culture.

At the end of the experiment, egg yolk fatty acids C14-C24 were determined. The C18:2 n6 values were statistically different between the control and experimental groups ($P < 0.05$). In addition, PUFA ($P = 0.06$), PUFA/SFA ($P < 0.03$), and n6 ($P = 0.06$) values were different between control and experimental groups. Yalçın et al. (2010) similarly reported that yeast autolysate supplementation decreased monounsaturated fatty acids (MUFA) and C18:1, but increased C14:0, C14:1, C20:0, C20:3n-3, and C20:5n-3. Yalçın et al. (2012) also reported that only C18:1 and MUFA levels increased, and the other fatty acid parameters were not affected by yeast culture supplementation.

The positive effects of yeast culture supplementation on the natural defense system of the host animal and on the biological regulation of its intestinal microflora and the

direct effect of the probiotic on health or the nutritional form of the probiotic have been previously reported (Shareef and Al-Dabbagh, 2009; Hassanein and Soliman, 2010). In the current study, total microbial counts, coliform bacteria, and yeast values were not altered in stool samples during the last week of the study. The lowest coliform bacteria value was found in the group fed 0.2% yeast culture supplementation. Fecal yeast count was higher in the yeast culture groups than in the control group. Gül et al. (2013) reported that the number of total bacteria was decreased, but the number of total yeast was increased in the intestine by yeast supplementation to laying hen diets. Another study (Hassanein and Soliman, 2010) reported that different levels (0.4, 0.8, 1.2, and 1.6%) of yeast supplementation to laying hen diets significantly reduced the number of total bacteria, especially in the 1.6% supplementation group. However, Wang et al. (2015) found that yeast supplementation increased the total number of bacteria in the intestine of laying hens.

Conclusions

Yeast culture supplementation, as a probiotic to laying hen diets, does not cause any health problems. It is thus suggested that the different levels of yeast supplementation (*S. cerevisiae*) in laying hen diets could increase the productivity of laying hens in terms of feed intake, egg weight, and egg Haugh unit.

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