Effect of dietary inclusion of live yeast (*Saccharomyces cerevisiae*) on growth performance, immune responses and blood parameters of broiler chickens

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This experiment was conducted to evaluate the effects of four levels (0, 0.1, 0.2 and 0.3%) of two forms(powdery and granular) of live yeast of *Saccharomyces cerevisiae(S.c)* on performance, humoral immune responses and blood parameters in broiler chickens. Two hundred and seventy three day-old commercial male broiler chicks were divided to 21 groups of 13 chicks each. Then each 3 groups were allocated to one of the 7 experimental diets, randomly. Data were collected for body weight, daily gain, feed intake and feed conversion ratio during the experimental period (0-49d of age). At the end of the experiment carcass yield and percentage of abdominal fat to live body weight were recorded. Antibody titers against Influenza disease virus (IDV) and Newcastle disease virus (NDV) in serum, on the 10th day of postimmunization(18, 28 and 38 d of age) were determined. On day 49, concentration of proteins, lipids in serum and heterophil to lymphocyte ratio (H/L) in chick's blood were evaluated. Results showed that use of live yeast *S.c* had no significant effect on performance of male broiler chickens. However, treatment containing 0.3% *S.c* (powdery) in compare to granular and control groups had a higher body weight (2792 g vs. 2711 and 2762 g), daily gain (56.2 vs. 54.5 and 55.6 g/d) and feed intake (105.3 vs. 101.5 and 103.6 g/d). The chickens fed powdery *S.c* in compare to granular and control groups had a lower abdominal fat and higher carcass yield. The use of *S.c* had no significant effect on antibody titers against IDV, but at 38d of age chicks fed 0.2% powdery *S.c* had a higher antibody titers against NDV than control group($P<0.05$). The chickens fed diets containing granular *S.c* had a lower H/L ratio in compare to powdery and control groups. The groups fed diets containing 0.1 and 0.2% powdery *S.c* had a lower serum cholesterol and higher serum HDL concentrations, respectively($P<0.05$). The results suggested that both granular and powdery forms of live yeast *Saccharomyces cervisiae* have not a growth stimulating effect in male broiler chicks. However in compare to granular type, addition of 0.1 or 0.2% of powdery form of live yeast *S.c* to diet can improve humoral immune responses, decrease serum lipids and suppress abdominal fat accumulation in broiler chickens.

Keywords: live yeast *Saccharomyces cerevisiae*; performance; immune responses; blood parameters; broilers.

Introduction

Dietary live yeast as *Saccharomyces cerevisiae* has been used as a fermenting agent in baking (Rose and Vijayalaskashmi, 1993), distilling (Watson, 1993) and brewing (Hammond, 1993) industries since ancient times, but today, there are many strains of the organism being used for different purposes (Onifade, 1998). Yeast has been known as a probiotic in feed animal (Saegusa *et al.*, 2004). Recently, in

animal feeding experiments, the use of supplemental yeast about 1 mg per kg has been found to yields various beneficial impacts (Onifade, 1998). However, the action mechanism of live yeast for improving performance is not fully understood, but there are two probabilistic explanations. The first, action of yeast is most probably supporting the growth of lactic acid bacteria. The second, a competitive exclusion of pathogenic bacteria by yeast and its products especially the cell wall component (Onifade, 1998). Yeast cell wall is containing chitin, mannan and glucan that have been known as immunostimulante (Li & Gatlin, 2003 ; Oliva–Teles & Goncalves, 2001; Rodriguez *et al* ., 2003; and Stewart, 1995). In recent years, four novel applications of yeast in animal production have emerged which are outside the conventional uses. These are: l- Yeast being used specifically for one of its metabolic products (the use of *Phaffia rhodozyma* carotenoids for egg-yolk color); 2- The ability of yeast to influence the normal microbial population within caecum; 3- The role of some yeasts as a modifier of the livestock gut microflora and to stimulator of immune system; 4- The use of *Saccharomyces cerevisiae*, when added to feed, to counteract aflatoxicosis in broiler chickens and ducklings. Models describing the effects of yeast on animal production are currently based on the ability of yeast strains to stimulate the growth and activities of gastrointestinal bacteria, but this stimulatory characteristic may not be common to all strains of yeast. Significant increases in phagocytic activity were observed when mannanoligosaccharides (MOS) incubated in peripheral blood from 3-month-old male Wistar rats. It is observed that birds fed MOS are better able to withstand the challenge (Stewart, 1995). A trial with inclusion of *Saccharomyces cerevisiae* in broiler diets containing 5ppm aflatoxin resulted in improvement of body weight and feed efficiency. Addition of 0.1% yeast culture reduced the adverse effects of the toxin (Stanley *et al., 1993;* Devegowda *et al.,* 1994). The present study was conducted to evaluate the effects of various levels of two forms (powdery and granular) of live yeast of *Saccharomyces cerevisiae* (*S.c*) on performance, immune responses and blood parameters in broiler chicks.

Materials and methods

Two hundred and seventy three day-old male broiler chicks (Ross 308) assigned to 21 pens of 7 treatment groups, randomly. The experimental design was completely random, consisting of three dietary levels (0.1, 0.2 and 0.3%) of each two forms (powdery and granular) of *S.c* and a control group (without yeast). Each treatment had three replicates of 13 birds. Chicks fed three basal of corn-soybean diets during three periods of 0-10, 11-28 and 29–49 d. The diets supplemented with amino-acids, minerals, and vitamins to meet all the Ross requirements (Ross 1999). The live yeast *S.c* (containing 1×10^9 CFU/g) was provided from Biosaf (granular, French) and Klar Maya (powdery, Iranian). Data were collected for body weight at 10, 28, and 49 d of age and for gain, feed intake and feed conversion ratio during periods of 0-10, 11-28 and 29-49 d. At the end of the experiment (49 d of age) some carcass traits like carcass yield and proportion of liver, bursa of fabricius, abdominal fat, and ceca to live body weight were recorded. Antibody titer against Influenza disease virus (IDV) and Newcastle disease virus (NDV) in serum, on the 10th of post vaccination (at 18, 28 and 38 d of age) were determined by hemagglutination inhibition (HI) test. The concentration of proteins (total protein, albumins and globulins) and lipids (cholesterol, triglycerides, low density lipoproteins (LDL) and high density lipoproteins (HDL)) in serum were determined at 49 d of age. Haematological indices such as erythrocytes (RBC), haemoglobin (Hb), haematocrit (PCV), white blood cell (WBC) and heterophil: lymphocyte (H/L) ratio also were determined on d 49. Data for all variables were subjected to an ANOVA analysis using the General Linear Models (GLM) procedure of SAS software (SAS Institute 1992) and means were considered different at $P<0.05$ using Duncan's multiple range test.

Results and Discussion

The results showed that use of live yeast of *S.c* (powdery and granular) had no significant effect on body weight, daily gain, feed intake, feed conversion (Table 1) and carcass charachteristics (Table 3). However treatments containing 0.3% powdery *S.c* in compare to granular and control groups had a higher body weight (2792 g vs. 2711 and 2762 g), daily gain (56.2 g/d vs. 54.5 and 55.6 g/d) and feed intake (105.3 g/d vs. 101.5 and 103.6 g/d). This observation disagree with one of the most consistent mechanisms of growth promotion by yeast culture in turkey and broiler chickens (Onifade and Babatunde,1996; Onifade, 1997).

Table 1. Effect of live yeast (*Saccharomyces cerevisiae*) on body weight, daily gain, feed intake and feed conversion of broiler chicks

Treatments	Body weight (g) (49 d of age)	Gain (g/d) (0-49 d of age)	Feed intake (g/d) (0-49 d of age)	Feed conversion (g/g)
control	2762.17	55.57	103.60 ab	1.87
0.1 % granular *S.c*	2780.87	55.93	100.93 ab	1.82
0.2 % granular *S.c*	2610.77	52.47	99.13 b	1.90
0.3 % granular *S.c*	2740.43	55.10	104.50 a	1.90
0.1 % powdery *S.c*	2775.77	55.80	105.13 a	1.88
0.2 % powdery *S.c*	2759.90	55.50	104.90 a	1.89
0.3 % powdery *S.c*	2792.17	56.17	105.30 a	1.87
Standard deviation	64.24	1.30	1.49	0.03

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

Chicks fed powdery *S.c* than granule and control groups had lower abdominal fat (1% vs. 1.23 and 1.2 %) and higher carcass yield (74.1 vs. 73.6 and 73.2 %). With increasing levels of powdery live yeast in diet, percentage of liver weight decreased. However, the use of live yeast of *S.c* (powdery and granular) increased percentage of ceca to live body weight.

Table 2. Effect of dietary live yeast (*Saccharomyces cerevisiae*) on carcass characteristics (as a proportion of live body weight) of broiler chicks at 49 days of age

Treatments	Carcass yield (%)	Abdominal fat (%)	Liver (%)	Bursa (%)	Ceca (%)
control	73.20 ab	1.20	1.93 ab	0.063 ab	0.45 b
0.1 % granular *S.c*	72.55 b	1.34	1.66 b	0.057b	0.50 ab
0.2 % granular *S.c*	72.22 b	1.27	2.05 ab	0.070 ab	0.64 a
0.3 % granular *S.c*	75.95 a	1.11	1.90 ab	0.058 b	0.56 ab
0.1 % powdery *S.c*	73.62 ab	0.96	2.13 a	0.083 a	0.58 ab
0.2 % powdery *S.c*	73.92 ab	0.93	2.08 a	0.055 b	0.62 ab
0.3 % powdery *S.c*	74.72 ab	1.11	1.84 ab	0.058 b	0.50 ab
Standard deviation	1.008	0.152	0.128	0.007	0.055

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

The effect of feeding 0.1, 0.2 and 0.3% *S.c* (powdery and granular) on antibody titer against Influenza disease virus (IDV) and Newcastle disease virus (NDV) of broiler chicks at 18, 28 and 38 d of age have shown in table 4. The use of *S.c* had no significant effect on antibody titer against IDV. However, at 18d of age chicks fed diet containing 0.1% powdery *S.c* had a higher antibody titer against IDV than chicks fed diet containing 0.3% powdery *S.c* (P<0.05) (4.17 vs. 2.17). On day 28, with increasing level of powdery *S.c* inclusion, antibody titer against IDV increased. The use of *S.c* (powdery and granular) had significant effect on antibody titer against NDV at 38d of age (P<0.05). Chicks fed diet containing 0.2% powdery *S.c* had a higher antibody titer against NDV than control group (4 vs. 2.5)(P<0.05). It seems that effectiveness of dietary inclusion of Sc. on humoral immune

response is considerable than its effect on performance of the chickens. Furthermore, the addition of powdery form of Sc. than granular and inclusion of 0.1 or 0.2% to diets than 0.3% elicited higher serum antibodies titers against IDV and NDV. Saegusa et. al(2004) suggested that *Saccharomyces cerevisiae* and *Candida albicans* in the intestine stimulate the host's mucosal immune system by interacting with intestinal epithelial cells.

Table 3. Effect of dietary live yeast *Saccharomyces cerevisiae* on antibody titers (log10) against IDV and NDV at different age of broiler chickens

Treatments	Anti IDV titers (log 10)			Anti IDV titers (log 10)		
	18d	28d	38d	18d	28d	38d
control	3.17 ab	2.67	3.83	2.67	1.83	2.50 bc
0.1 % granular *S.c*	3.67 ab	2.50	3.50	2.50	1.83	1.67 c
0.2 % granular *S.c*	3.83 a	3.33	3.17	2.50	2.67	2.50 bc
0.3 % granular *S.c*	3.17 ab	2.33	3.67	2.17	1.17	2.83 ab
0.1 % powdery *S.c*	4.17 a	2.67	3.67	3	2.17	3.50 ab
0.2 % powdery *S.c*	3.67 ab	3.17	3.83	3.33	2.67	4 a
0.3 % powdery *S.c*	2.17 b	3.33	3.83	1.83	2	3.83 ab
Standard deviation	0.49	0.46	0.38	0.49	0.45	0.44

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

The use of live yeast *S.c* (powdery and granular) had no significant effects on haematological indices like RBC, WBC and PCV, but differences between treatments were significant for Hb level and H/L ratio (P<0.05) (Table 4). However, RBC, Hb and PCV were higher in chickens fed diet containing 0.2% *S.c* (granular). Onifade et. al (1999) and Onifade (1997) also reported a positive correlation between dietary levels of S.c with the haematological indices like , RBC, WBC and PCV in rabbit and broiler chickens. They suggested that theses correlations may be an additional mechanism growth promotion by supplemental yeast. In our experiment, all yeast-fed chicks in compare to control diet had a lower H/L ratio by the higher populations of lymphocytes than control diet. However, only chickens fed 0.3% granular *S.c* had a significant lower H/L ratio in compare to control group. The same trend of lymphocyte populations and serum antibody levels may be indicative of higher activity of humoral immune responses in chicks fed yeast supplemented diets.

Table 4. Effect of live yeast (*Saccharomyces cerevisiae*) on haematological indices of broiler chicks at 49 days of age

Treatment	RBC[1] (10^6 ul^{-1})	Hb [2] (g/dl)	PCV [3] (%)	WBC[4] (10^6 ul^{-1})	H /L [5]
control	2.61	13.43 ab	28.90	6233	0.64 a
0.1 % granular *S.c*	2.42	13.17 ab	28.07	7667	0.25 b
0.2 % granular *S.c*	2.78	14.10 a	30.60	5600	0.43 ab
0.3 % granular *S.c*	2.74	14.10 a	30.30	9100	0.23 b
0.1 % powdery *S.c*	2.39	12.27 b	29.57	8567	0.51 ab
0.2 % powdery *S.c*	2.45	13.03 ab	26.67	4667	0.46 ab
0.3 % powdery *S.c*	2.47	13.07 ab	27.03	6367	0.49 ab
Standard deviation	0.13	0.52	1.75	1910	0.10

[a,b] Means within a column followed by different superscripts differ significantly (P<0.05).
1-Red Blood Cell 2- Haemoglobin 3-Haematocrit 4-White Blood Cell 5-Heterophil: Lymphocyte ratio

The results of clinical chemistry as a further measure of the response of chickens to nutritional regimens are depicted in Table 5. Serum concentrations of albumin, total protein, triglyceride, HDL, LDL and A/G ratio were not significantly affected by the treatments. Serum total protein and albumin have been reported to be directly responsive to protein intake and quality (Eggum, 1989; Onifade and Abu, 1998). Diet containing 0.2 % powdery *S.c* increased serum HDL concentration. With increasing

level of granular *S.c* in experimental diet, serum LDL and cholesterol concentration increased. On the other hand, in chickens fed diet containing 0.1% *S.c* in powder form decreased serum cholesterol concentration compare with control group (P<0.05). Reduction in circulating cholesterol and LDL with supplemental yeast was remarkable and agree with the results of other researchers (Onifade et al, 1999; Onifade, 1997) that the addition of innocuous micro organisms including yeast to diet of rabbit and broiler chickens decrease serum cholesterol, triglycerides, phospholipids and abdominal fat. Overall, it can be concluded from this study that the addition of both granular and powdery forms of live yeast *Saccharomyces cervisiae* had not a growth stimulating effect in male broiler chickens. In addition, it seems that dietary inclusion of live yeast (particularly in powder form) could be an effective stimulator of humoral immune responses in broiler chickens. However, more studies would be necessary to obtain evidence on specific advantages of supplementing yeast and determining economically optimum dietary concentration.

Table 5. Effect of live yeast (*Saccharomyces cerevisiae*) on serum biochemical indices of broiler chicks at 49 days of age

treatment	Albumin (%)	A/G[1]	Total protein (g/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL[2] (mg/dl)	LDL[3] (mg/dl)
control	37	0.59	4.70	122.50 abc	79.50	100.17	18.27ab
0.1 % granular *S.c*	36.20	0.59	3.90	116 bc	86.17	94.17	11.17 b
0.2 % granular *S.c*	38.67	0.64	4.40	121.50 bc	79.83	98.17	16.80ab
0.3 % granular *S.c*	37.50	0.61	3.77	133.67 a	81.50	99.17	24.07 a
0.1 % powdery *S.c*	34.40	0.52	4.70	111.50 c	68	89.50	14 b
0.2 % powdery *S.c*	31.17	0.46	4	126.33 ab	85.17	103.17	15 b
0.3 % powdery *S.c*	35.07	0.54	4.03	115.83 bc	78.67	87	13.87 b
Standard deviation	2.70	0.07	0.41	3.64	8.83	6.62	2.57

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

1-Albumin to Globulin ratio 2-High Density Lipoprotein 3-Low Density Lipoprotein

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