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The Effects of Supplemental *Saccharomyces cerevisiae* and Phytase on Growth Performances and Plasma Biochemical Parameters of Broiler Chickens

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Abstract: *Saccharomyces cerevisiae* and phytase can be used as a feed supplement in combination or alone for broiler feeding. An experiment was performed to study the effect of supplemental different ratio *S. cerevisiae* and phytase combination on growth performances, plasma biochemical parameters and plasma enzyme activities of broiler chickens. Animal were feed *ad libitum* through the study. Sixty hundred 1 day-old broiler chicks were randomly selected and distributed into eight groups as control (C); phytase (P) (added 200 g Pyhase to 1000 kg of diet); *S. cerevisiae* (Y1) (added 0.1% *S. cerevisiae* of the diet); Y1+P (combination of group Y1 and P); *S. cerevisiae* (Y2) (added 0.2% *S. cerevisiae* of the diet); Y2+P (Combination of Y2 and P); Y4 (added 0.4% *S. cerevisiae* of the diet); Y4+P (Combination of Y4 and P) respectively. At the end of the study, blood samples were collected from 15 randomly selected broiler chickens from each group through the brachial vein on the 42nd day of the experiment. Plasma were separated and used for measurement of plasma biochemical parameters and enzyme activities. *S.cerevisiae* alone and combination of Phytase and *S. cerevisiae* increased body weight and body weight gain of broiler, especially in 6th week, except in group Y4. Although, the broiler chickens supplemented with just phytase or/and *S. cerevisiae* and their combination had lower plasma triglyceride, total cholesterol, LDL-cholesterol, glucose, calcium and GGT enzyme activity , they had higher HDL-cholesterol, phosphorus and ALT enzyme. Moreover, supplementation with just phytase or/and *S. cerevisiae* and their combination did not change the hematocrit, plasma total protein and AST enzyme levels of the broiler chickens. These results demonstrated that phytase and *S. cerevisiae* alone or/and their combination improved growth performance and body weight gain of the broiler chickens. So, *S.cerevisiae* and phytase combination may be used as an growth enhancer. However, more studies would be necessary to obtain the effects of supplementing yeast and phytase combination on growth.

Key Words: Biochemical parameters, Broiler chicken, Growth performance, Enzyme activities, *S. cerevisiae*, Phytase.

Broiler Tavuk larda Yeme İlave Edilen Saccharomyces cerevisiae ve Fitaz'ın Büyüme Performansı ve Plazma Biyokimyasal Parametreleri Üzerine Etkileri

Özet: *Saccharomyces cerevisiae* ve fitaz ayrı ayrı olarak broiler tavuklarda yem katkısı amacıyla kullanılmaktadır. Bu araştırma, farklı oranlarda *S. cerevisiae*'nin ve fitaz kombinasyonunun broiler tavuklarda büyüme performansı, plazma biyokimyasal parametreleri ve plazma enzim aktiviteleri üzerindeki etkisini değerlendirmek için yapıldı. Çalışma boyunca hayvanlar *ad libitum* beslendi. 1 günlük yaşta 600 adet broiler tavuk rastgele seçildi ve 8 gruba sırasıyla şu şekilde ayrıldı: Kontrol (C); Fitaz (P) (200 g Fitaz 1000 kg yeme ilave edildi); *S. cerevisiae* (Y1) (% 0,1 oranında *S. cerevisiae* yeme ilave edildi); Y1+P (grup Y1 ve P'nin kombinasyonu); Y2 (% 0,2 oranında *S. cerevisiae* yeme ilave edildi); Y2+P (grup Y2 ve P'nin kombinasyonu); Y4 (% 0,4 oranında

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S. cerevisiae yeme ilave edildi); Y4+P (grup Y4 ve P'nin kombinasyonu). Çalışmanın sonlandırıldığı 42. günde, her gruptan rastgele seçilen 15 adet hayvanın brahial veninden kan örnekleri alındı. Kan örneklerinin plazması ayrıldı ve biyokimyasal parametreler ve enzim aktivitelerinin ölçülmesi için kullanıldı.

S. cerevisiae, tek başına ve S.cerevisiae ve fitaz kombinasyonları, Y4 grubu hariç, özellikle 6. haftada tavuklarda canlı ağırlık ve canlı ağırlık kazanımını arttırdı. Sadece fitaz ve/veya *S. cerevisiae* ve kombinasyonlarının verildiği tavuklarda plazma trigliserid, total kolesterol, LDL_kolesterol, glukoz, kalsiyum ve GGT enzimi azalırken, hayvanlarda yüksek düzeyde HDL-kolesterol, fosfor ve ALT enzimi tespit edildi. Diğer taraftan, tavuklarda sadece fitaz ve/veya *S. cerevisiae* ve kombinasyonlarının yem katkısı olarak kullanılması, hematokrit, plazma protein ve AST enzim değerlerini değiştirmedi. Sonuçlar fitaz ve *S. cerevisiae*'nın yalnız ve/veya kombinasyonlarının yem katkısı olarak kullanılmasının büyüme performansı ve canlı ağırlık kazanımını arttırdığını göstermektedir. Böylece, *S.cerevisiae* ve fitaz kombinasyonları büyümeyi arttırıcı olarak kullanılabilir. Ancak, maya ve fitaz kombinasyonlarının büyümedeki etkisinin ortaya konması için daha fazla çalışmanın yapılması gerekmektedir.

Anahtar Sözcükler: Biyokimyasal parametreler broiler tavuk, büyüme performansı, enzim aktiviteleri, Fitaz, *S. cerevisiae*.

Introduction

The feed additives like antibiotics and hormones were used in poultry for many years. Nowadays, natural feed additives such as probiotics have an important role in improving performances in poultry. Many kinds of probiotics, especially the live yeast *S. cereivisae*, have been used for chickens. *S. cereivisae* is an important protein source for animals. *S. cerevisiae* contains biologically valuable proteins, vitamin B-complex and important trace minerals.

Eckles and Williams[12] first reported the use of *S. cerevisiae* as a growth promoter for ruminants. Also, it was reported that beta glucan and mannan oligosaccharide obtained from *S. cerevisiae* enhanced the body weight gain and feed conversation ratio[19,22,23,30,38]. There are some suggested mechanisms about yeast effects on growth performance. Yeast supports the digestive process by inhibiting the harmful bacteria in the intestinal mucosa and helps the digestion of foods, and thereby enhances the growth and plazma protein values[16,17,34]. The gastrointestinal flora is important for performance of poultry[12,56]. *S. cerevisiae* has been survived in the gastrointestinal tract while eliminating the pathogenic bacteria[4]. It has been suggested that probiotics binds to bile acids which results in a reduced serum cholesterol value[15]. There are many controversal studies about the effect of *S. cerevisiae* on serum lipid profile in animals. Although some of studies showed cholesterol reduction[34,46], the others demonstrated no benefits[17,33]. Other beneficial factors of *S. cerevisiae* include the enhancement of phosphorus availability[7] and utilization by animals[13,53]. Also, serum calcium has important issues in poultry nutrition for bone mineralization. Some researchers[6,18] have indicated the increase in mineral retention and bone mineralization of chickens due to mannanoligosaccaride in *S. cerevisiae*. Phosphorus and calcium which may led to improved appetite of the chickens and hence improved feed intake and growth performance was reported by Akhavan-Salamat et al.[2].

Phytase is an enzyme that participates in the diet and recommends due to its effect of breaking down phytates in foods to improve the efficiency of diets. It's also enhances phosphorus utilization[50]. Phosphorus is important for the development of the skeletal system, and is involved in carbohydrate and fat metabolism. There are several studies which indicated that phytase supplementation increased the body weight gain, feed intake and feed efficiency in broiler chickens[42,43,49,51]. The effects of phytase supplementation on mineral[5,48], protein and amino acids[20], carbohydrates[21] and energy[37] was reported in many studies. Phytase reduces the inclusion of higher cost of phosphorus sources[50]. Erkek and Unlu[14] stated that the addition of phytase to diets for chickens improved the digestibility of some minerals and nutrients which not digested in normal condition, and thereby reduced these nutrients in soil and minimized the environmental pollution due to phosphorus.

Although some studies have been performed in the growth performance of phytase and *S.cerevisiae* in animals[2,13,42,43], the combined impression of phytase and *S.cerevisiae* have not been addressed. Considering these above data, current study was aimed to evaluate the combined effects of phytase and *S. cerevisiae* on growth performances and plasma biochemical parameters of broiler chickens.

Material and Methods

Animals, Groups and Feeding: The experiment was carried out on broiler chicks (n: 600) purchased from CP Inc. Com, Bursa, Turkey. The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of Uludag University Animal Experimentation Local Ethics Committee (Approval No: 2013-02/06).

Sixty hundred 1 day-old chicks were randomly selected and distributed into eight groups of seventy five day-old chicks each. *S. cerevisiae* live yeast culture (YeaSacc[1026]: $1x10^9$ CFU g^{-1}, Alltech, Nicholasville) and Phytase (Allzyme SSF, Alltech, Nicholasville) were supplemented with basal diet. All diets were formulated to provide 3100 kcal of ME/kg and to meet the amino acid ratios and all other nutrients as suggested by the NRC[28]. The compositions of the diets are presented in Table 1. Depending on the experimental design, groups are: I. Control (C); II. Phytase (P) (added 200 g Pyhtase to 1000 kg of diet); III. *S. cerevisiae* (Y1) (added 0.1% Saccharomyces cerevisiae of the diet); IV. Y1+P (combination of group Y1 and P); V. S. cerevisiae (Y2) (added 0.2% *Saccharomyces cerevisiae* of the diet), VI. Y2+P (Combination of Y2 and P); VII. Y4 (added 0.4% *Saccharomyces cerevisiae* of the diet); VIII. Y4+P (Combination of Y4 and P) respectively. In the groups, *S.cerevisiae* and phytase rations were chosen previously reported studies[24,31,45].

The chicks were housed in an environmentally controlled poultry house with the floor covered with wood shavings and kept dry throughout the study. Feed and water were provided *ad libitum*. The feeding was lasted 42 days. The animals were fed commercial broiler starter diet (CP Inc. Com, Bursa, Turkey) for the first 20 days and pelleted grower diet (CP Inc. Com, Bursa, Turkey) from 21 to 35 days of age, and finisher diet (CP Inc. Com, Bursa, Turkey) from 36 to 42 days of age. Ingredient and nutrient compositions of diets are shown in Table 1.

Table 1. The nutrient composition of the experimental diets.

Tablo 1. Yemin besinsel içeriği.

Nutrient content	Starter (0-20 d)	Grower (21-35 d)	Finisher (36-42 d)
ME (kcal/kg)	3100	3100	3100
Crude protein (%)	22.50	21.50	20.00
Crude fibre (%)	3.40	3	3
Oil (%)	6	5.20	5
Ash (%)	5	6	6
Lysine (%)	1.40	1.30	1.20
Methionine (%)	0.60	0.50	0.44
Calcium (%)	0.80	0.75	0.70
Vitamin mineral premix*	27.40	27.40	26.70

* Provided the following per kg of diet: vitamin A 10.000IU, vitamin D3 5000 IU, vitamin D3 4000 IU (only for finisher diet), vitamin E 75 mg, vitamin E 50 mg (only for finisher diet), Phosphorus 7000 mg, Sodium 2000 mg, Manganese 120 mg, Zinc 100 mg,
Selenium 0.30 mg, Iron 40 mg, Iodine 1.25 mg, Copper 16 mg.

* Kg başına yem içeriği: 10.000IU A vitamini, 5000 IU D3 vitamini, 4000 IU D3 vitamini (sadece bitirme yeminde), 75 mg E vitamini, 50 mg E vitamini (sadece bitirme yeminde), 7000 mg Fosfor, 2000 mg Sodyum, 120 mg Mangan, 100 mg Çinko, 0.30 mg Selenyum, 40 mg Demir, 1.25 mg İyot, 16 mg Bakır.

Measurements: Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were recorded weekly for all treatment groups. Feed conversion ratio was calculated according to following formula: total feed intake / total weight gain for each period.

Blood samples were collected from 15 randomly selected broiler chickens from each group through the brachial vein on the 42nd day of the experiment. For hematocrit (HT) measurement blood samples were collected in micro-hematocrit tubes and centrifuged at 10000 RPM for 5 min. 3 ml blood samples for biochemical parameters were collected with heparinized tubes. Plasma was obtained by centrifugation at 3000 RPM in 5 minutes. Plasma concentrations of total protein, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose, phosphorus, calcium and activities of alkaline phosphatase (ALP), alanin aminotransferaz (ALT), aspartate aminotransferaz (AST) and gamma-glytamyl transpeptidase (GGT) were investigated by enzymatic colorimetric method with "Clima MC15" auto analyzer (RAL, Barcelona, Spain).

Statistical Analysis: Statistical analyses were performed with SPSS[52]. Data were tested

for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. Data are stated as mean±standard error of the mean (SEM). One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at P<0.05. If the difference between groups was provided to be significant (P<0.05), differences evaluated group by Tukey's test[11]. On the other hand, in non-homogenous groups, differences between means were analyzed by Kruskal Wallis and following Mann Whitney U test between groups one by one[9].

Results

Growth Performance: The broiler chicks had almost 2 % mortality rate over the whole period of the experiment. There is no significant difference in mortality rate between the groups (data not shown). The body weight, body weight gain, feed intake and feed conversation rate of broilers is shown in Table 2 and Table 3, respectively. Broilers supplemented with different concentration of *S. cerevisiae* and phytase and different concentration of yeast combination had greater body weight (P<0,05; Table 2) in all groups and body weight gain (P<0,05; Table 3) in groups Y1+P, Y2, Y2+P and Y4+P than control broilers at 6 weeks of age. Accord-

Table 2. The effect of supplemental S. cerevisiae and phytase on body weight of broiler chickens

Tablo 2. Broiler tavuklarda S. cerevisiae ve fitaz katkısının canlı ağırlığa etkisi

	Body Weight							
Weeks	C	P	Y1	Y1+P	Y2	Y2+P	Y4	Y4+P
0	45.37±0.40	45.70±0.44	45.93±0.43	45.77±0.40	45.83±0.37	46.17±0.46	44.95±0.37	44.80±0.36
1	145.51±1.88	159.20±2.15*	163.06±1.88*	163.78±2.02*	161.27±1.62*	160.66±2.08*	156.82±1.84*	161.46±2.13*
2	431.64±5.13	514.11±6.42*	530.79±6.03*	533.30±5.95*	527.65±6.03*	521.70±6.62*	518.54±5.82*	529.07±6.23*
3	880.66±10.32	1002.28±13.19*	1173.87±18.80*	1218.24±14.29*	1038.92±10.60*	1019.51±14.37*	1031.49±9.82*	1036.24±10.41*
4	1475.62±17.62	1600.50±21.18*	1635.75±21.15*	1654.76±19.44*	1639.35±16.36*	1618.30±19.94*	1631.59±15.25*	1621.41±18.92*
5	2140.05±23.99	2192.67±34.25*	2228.52±23.80*	2289.97±23.85*	2219.20±22.55*	2231.89±22.19*	2233.78±25.98*	2221.36±24.77*
6	2701.53±30.32	2751.50±40.66	2701.37±31.45	2828.95±27.21*	2788.24±28.83*	2785.32±37.71*	2754.86±23.45*	2775.12±32.58*

* P<0.05

** Note: Means with different superscripts in the same row differ at P< 0.05.

** Not: Gruplar arasında farklılıklar P<0.05'de gösterilmiştir.

Table 3. The effect of supplemental S. cerevisiae and phytase on body weight gain, feed intake and feed conversation ratio of broiler chickens

Tablo 3. Broiler tavuklarda S. cerevisiae ve fitaz katkısının canlı ağırlık artışına, yem tüketimine ve yemden yararlanma oranına etkisi

	Body Weight Gain							
Weeks	C	P	Y1	Y1+P	Y2	Y2+P	Y4	Y4+P
1	100.14±1.90	113.50±2.18*	117.13±1.90*	118.01±2.04*	115.44±1.72*	114.49±1.19*	111.87±1.90	116.66±2.17*
2	386.27±5.16	468.41±6.41*	484.87±6.03*	487.52±5.88*	481.82±6.03*	475.53±6.72*	473.59±5.87	484.27±6.26*
3	835.29±10.29	956,58±13.14*	1127.95±18.73*	1172.47±14.39*	993.09±10.66*	973.33±14.29*	986.53±9.77*	991.44±10.47*
4	1430.25±17.61	1554.80±21.19*	1589.83±21.11*	1608.98±19.49*	1593.52±16.35*	1572.13±19.97*	1586.64±15.18	1576.61±18.90*
5	2094.69±24.02	2146.97±34.20*	2182.59±23.84*	2244.20±23.94*	2173.37±22.53*	2185.72±22.11*	2188.83±25.95	2176.56±24.75*
6	2656.17±30.36	2705.80±40.63	2655.44±31.51	2780.17±27.15*	2742.41±28.86*	2739.14±37.72*	2709.91±23.42	2730.32±32.51*
	Feed Intake							
Weeks	C	P	Y1	Y1+P	Y2	Y2+P	Y4	Y4+P
1	4120.0±167.73	4168.00±72.77	4330.67±187.28	4208.00±101.13	4258.00±113.78	4411.33±81.14	4294.67±66.64	4708.67±71.19
2	11834.67±580.47	12256.67±116.60	11965.33±485.57	11922.00±232.29	12784.00±313.58	12519.33±267.13	12196.67±307.76	12891.33±137.36
3	15296.00±153.70	16352.67±437.37	17178.67±396.49	16997.33±300.49	17260.00±141.00	17155.33±217.97	16786.00±607.15	17030.67±108.55
4	27786.67±536.69	25870.67±569.32	26552.00±569.32	28156.67±418.56	27306.67±399.53	25946.00±522,65	26206.67±531.23	26798.67±559.13
5	47736.00±309.07	50152.67±305.08	50152.67±305.08	51318.00±293.89	50511.33±317.93	51168.00±509.50	49896.00±546.24	49597.33±401.62
6	30046.67±283.35	28257.33±727.01	29257.33±727.01	31667.33±332.69	31217.33±437.01	31092.00±879.43	30400.67±854.61	31002.00±527.07
	Feed Conversation Ratio							
Weeks	C	P	Y1	Y1+P	Y2	Y2+P	Y4	Y4+P
1	0.025±0.0014	0.027±0.0006	0.027±0.0006	0.028±0.0015	0.027±0.0008	0.026±0.0005	0.025±0.0003	0.025±0.0005
2	0.033±0,0017	0.038±0,0003	0.040±0,0012	0.041±0,0005	0.038±0,0008	0.039±0,0008	0.039±0,0015	0.038±0,0003
3	0.055±0.0008	0.058±0.0003	0.065±0.0060	0.069±0.0097	0.058±0.0003	0.058±0.0003	0.059±0.0012	0.058±0.0003
4	0.052±0.0046	0.060±0.0011	0.060±0.0006	0.057±0.0008	0.058±0.0008	0.060±0.0018	0.060±0.0017	0.059±0.0007
5	0.070±0.0006	0.072±0.0023	0.074±0.0019	0.070±0.0013	0.070±0.0012	0.070±0.0017	0.071±0.0019	0.070±0.0014
6	0.089±0.0013	0.091±0.0017	0.091±0.0015	0.088±0.0023	0.088±0.0020	0.088±0.0013	0.089±0.0023	0.088±0.0015

* P<0.05

** Note: Means with different superscripts in the same column differ at P < 0.05.

** Not: Gruplar arasında farklılıklar P<0.05'de gösterilmiştir.

ing to results, phytase and *S. cerevisiae* supplementation together increased the growth performance of the broiler. No significant differences were observed in feed intake and feed conversation rate (Table 3) between the groups.

Blood Constituents: The results of plasma total protein, total cholesterol, LDL- and HDL-cholesterol, glucose, phosphorus, calcium concentration were summarized in Table 4. Also Hematocrit values, AST, ALT and GGT enzyme activities of supplemented broiler were shown in Table 5. There were no significant differences in hematocrit values and plasma total protein concentrations among all groups (Table 5). However, the triglyceride, total cholesterol and LDL-cholesterol values were found to be lower in phytase and yeast supplemented groups compared to control group (P<0.05), the HDL-cholesterol values of chickens in Y1+P, Y2, Y2+P, Y4 and Y4+P groups were significantly (P<0.05) higher than the control group (Table 4). Plasma glucose levels of broilers in Y2, Y2+P, Y4 and Y4+P groups were lower than the C, P and Y1 groups (Table 4). The broilers in Y1+P, Y2, Y2+P, Y4 and Y4+P groups had significantly higher plasma phosphorus level and lower plasma calcium level compared to control group broiler (Table 4). Although the value of GGT activities in plasma was significantly decreased in Y1+P, Y2, Y2+P, Y4 and Y4+P groups compared to control group (P<0.05), there was a significant increase in plasma ALT activities in the chickens at Y4 and Y4+P groups compared to control (Table 5). On the other hand, there was no significant difference in AST activity levels among all experimental groups compared to control group (Table 5).

Table 4. The effect of supplemental S. cerevisiae and phytase on plasma biochemical parameters of broiler chickens

Tablo 4. Broiler tavuklarda S. cerevisiae ve fitaz katkısının biyokimyasal parametreler üzerine etkisi

Groups	T. Protein	Triglyceride	T. Choles.	Parameters LDL-C	HDL-C	Glucose	Phosphorus	Calcium
C	3.29 ±0.11	60.77±1.39	143.43±2.67	42.91±2.17	103.13±3.91	214.42±4.67	5.78±0,27	13.78±0,32
P	2.96±0.17	59.93±2.24	140.64±4.84	36.40±2.15	109.69±5.88	208.33±4.66	5.91±0,25	14.89±0,27
Y1	3.25±0.12	60.88±1.77	140.90±3.19	34.29±1.68*	108.23±3.22	201.07±4.82	6.19±0,31	13.25±0,54
Y1+P	3.69±0.20	54.77±1.46*	142.57±4.98	30.66±1.62*	125.50±3.80*	203.00±6.88	7.37±0,23*	12.01±0,22*
Y2	3.17±0.11	55.46±1.35*	143.79±8.94	23.41±1.62*	112.86±5.28	189.40±5.11*	7.91±0,15*	11.26±0,24*
Y2+P	3.46±0.22	56.38±1.40*	143.21±2.98	28.80±1.35*	125.53±3.68*	190.00±6.72*	7.02±0,31*	11.02±0,27*
Y4	3.12±0.15	55.00±1.65*	137.01±3.95*	31.29±1.36*	132.64±3.79*	173.57±4.96*	6.92±0,28*	10.35±0,31*
Y4+P	3.20±0.12	51.36±2.74*	136.01±4.15*	27.87±1.12*	125.43±3.14*	158.73±2.41*	6.66±0,22*	9.15±0,22*

* P<0.05

** Note: Means with different superscripts in the same row differ at P < 0.05.

** Not: Gruplar arasında farklılıklar P<0.05'de gösterilmiştir.

Table 5. The effect of supplemental S. cerevisiae and phytase on hematocrit and liver enzymes of broiler chickens*

Tablo 5. Broiler tavuklarda S. cerevisiae ve fitaz katkısının hematokrit ve karaciğer enzimlerine etkisi*

Groups	Parameters HT	ALT	AST	GGT
C	29.14±0.39	8.62±0.67	159.77±7.53	16.07±1.05
P	30.01±0.46	8.43±0.80	157.69±8.32	15.53±1.01
Y1	30.01±0.67	10.60±0.58	159.28±12.34	15.21±0.71
Y1+P	30.06±0.49	8.13±0.41	157.00±11.89	13.21±0.76*
Y2	29.45±0.85	7.67±0.42	157.93±9.56	11.13±0.54*
Y2+P	29.06±0.58	10.00±0.78	151.86±9.11	10.07±0.67*
Y4	29.53±0.61	13.64±0.80*	154.93±5.98	11.79±0.52*
Y4+P	30.01±0.82	13.33±0.62*	158.29±6.70	12.79±0.56*

* P<0.05

* Note: Means with different superscripts in the same row differ at P < 0.05.

* Not: Gruplar arasında farklılıklar P<0.05'de gösterilmiştir.

Discussion and Conclusion

The results of the present study indicate that different concentration of *S. cerevisiae* and phytase and different concentration of the yeast combination increased body weight and body weight gain of broiler, especially in 6th week. While the broiler fed with phytase, different concentration of *S. cerevisiae* and phytase and different concentration of the yeast combination had lower plasma triglyceride, total cholesterol, LDL-cholesterol, glucose, calcium and GGT enzyme, they had higher HDL-cholesterol, phosphorus and ALT enzyme. Phytase, different concentration of *S. cerevisiae* and phytase and different concentration of the yeast combination had no effect on the hematocrit, plasma total protein and AST enzyme levels of the broilers.

In the present study, the broiler fed with different concentration of *S. cerevisiae* (0.2% and 0.4%) and phytase and different concentration of the yeast combination supplement obtained more body weight and body weight gain significantly. These results demonstrated that yeast supplementation or yeast and phytase combination had positive effect on body weight and weight gain. It was also reported that yeast reduced the feed conversation ratio, resulting in increased weight gain[28]. In the present study, although not statistically significant, the feed conversation ratio tended to be decrease in 6th week. It was indicated that *S. cerevisiae* cell wall components beta glucan and mannan oligosaccarides may be responsible for the positive improvement of the growth performance[40]. It was also shown that mannan oligosaccharide had a significant improvement in feed conversion and body weight gain in broiler chickens[10]. An increased weight gain and feed efficiency with phytase has been also reported before[53,44]. According to results, although some of studies have been performed in the growth performance of phytase and *S.cerevisiae* in animals[10,35,40,44], the combined effects of phytase and *S.cerevisiae* have not been addressed. In the present study we, first time, shown the synergistic effect of phytase and *S. cerevisiae* with supplementing those together. Nevertheless, previous studies reported that *S. cerevisiae* expressed genetically phytase activity and had a positive effect in the gastrointestinal tract[32,57]. Those results seem correlated with our finding.

In current study, the broiler fed with phytase alone, different concentration of *S. cerevisiae*, and phytase with different concentration of the yeast combination had lower plasma triglyceride, total cholesterol, LDL-cholesterol, glucose, calcium and GGT enzyme, they had higher HDL-cholesterol, phosphorus and ALT enzyme. *S. cerevisiae* cell wall component, the beta glucan, had a cholesterol lowering effect was documented by some researchers[15,34,36]. These researchers suggested that yeast may regulate the serum cholesterol concentrations by deconjunction of bile acids. It was also reported that dietary phytase was able to affect lipase activity and lipid metabolism of broiler chickens, especially increasing HDL-cholesterol[25]. These finding shows that, interestingly, the inclusion of phytase or yeast or their combination could modify the levels of most lipids profiles of chickens, suggesting that the supplements might contribute to the lipid metabolism and body fat synthesis.

Although, there were no significant changes in plasma protein levels in all groups compared to control, the plasma protein values in groups Y1+P, Y2+P and Y4+P were higher than P group. This may be due to combined effect of yeast and phytase on plasma protein metabolism. It is documented that yeast can produce phytase that is needed to hydrolyze phytate into inorganic phosphate[47]. Also, Cowieson et al.[8] reported that phytase improves the bioavailability of phosphorus, calcium and amino acids.

In recent study, plasma glucose level in group Y2, Y2P, Y4, Y4P were lower than control. This may be due to affect of phytase on glucose absorption. It was reported that dietary phytate may affect the starch digestion and blood glucose response in humans[55]. Phytic acid may reduce the digestive carbohydrases by binding to the digestive enzymes or dietary protein that is closely associated with starch. The reduced glucose absorption could explain the reduced energy digestibility by phytic acid[54]. On the other hand, some of studies showed serum glucose increase by addition of *S. cerevisiae*[39,45], the others documented that *S. cerevisiae* has no benefits on serum glucose level[1].

The mineral concentrations in plasma in chickens dependent on mineral content of diets and absorbtion of the minerals in digestive system[27]. Mineral homeostasis is regulated by both neural and humoural mechanisms. Scott[41] reported that this is very important for the growth rate of broiler chickens skeletal system. Because

skeletal weakness is important both an economic and an animal welfare concern. In this study, the increased dietary level of *S. cerevisiae* increased plasma phosporus content and decreased plasma calcium concentration in the group Y1+P, Y2, Y2+P, Y4 and Y4+P compared with the control ($P<0.05$), as shown in Table 4. Aluwong et al.[3] had the same results of plasma calcium in broiler chickens. Also, Akhavan-Salamat et al.[2] showed that yeast increases the bone calcium level that improves the force of bones in chickens.

The enzymes, AST, ALP, ALT and GGT are located intracellularly in the body including liver, heart, kidney and osteoblast etc. Their levels in blood are increased when there is membrane damage in these cells, especially in liver. On the other hand, if their levels are low, it means no clinical significance. In this study, the average values of GGT in plasma was significantly decreased in all groups compared to control group ($P<0.05$), although there were significant increases in plasma ALT in group Y4 and Y4+P compared to control. The increase in activities of these enzymes may be due to damage in liver cells, but not very abnormal. Yalcin et al.[58] reported an abnormal increase of these enzymes and liver damage in laying hens with addition of *S. cerevisiae*. Also, Mannan et al.[26] reported that addition of *S. cerevisiae* caused significant increase in serum AST and ALT activities may be due to animal species and probiotic interventions. Nevertheless, there were no significant changes in AST values in all groups compared to control in this study.

In summary the results of the present study suggest that dietary phytase and *S. cerevisiae* can increase body weight, and body weight gain of the broiler and modify the levels of lipids and proteins, balance of phosphorus and calcium ratio, and biochemical activity of liver enzymes for broiler chickens. It was concluded that phytase may improve the *S.cerevisiae* activity and thereby affect the growth performance of animals.

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