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EFFECTS OF *SACCHAROMYCES CEREVISIAE*, *THEPAX* AND THEIR COMBINATION ON BLOOD ENZYMES AND PERFORMANCE OF JAPANESE QUAILS (*COTURNIX JAPONICA*)

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ABSTRACT

The present study was conducted to investigate the influence of probiotic, prebiotic and their combination as synbiotic on broiler performance, organs weight and blood enzymes of Japanese Quails. Total of 192 one-day old Japanese quail chicks were randomly assigned to 4 treatments and 4 replicates. The experimental diets consisted of a basal diet (A) without additive (Control), (B) 2 g/kg *Saccharomyces cerevisiae* (probiotic), (C) 1 g/kg *Thepax* (prebiotic) and (D) 1 g/kg *Saccharomyces cerevisiae*+ 0.5 g/kg *Thepax* (synbiotic) added to the basal diet. Birds fed probiotic and prebiotic showed elevation in body weight compared to other groups (P<0.05). Feed intake of birds fed probiotic and prebiotic were higher than control group (P<0.05). Birds fed on probiotic and prebiotic exhibited a better feed conversion ratio compared to synbiotic fed and control groups (P<0.05). Dressing percentage of birds fed probiotic (74.9±2), prebiotic (76.2±3) and synbiotic (73.7±1) were more than control group (70.1±2) (P<0.05). The both male and female birds fed additives showed decrease in liver weight (P<0.05). The relative weight of heart was decreased in the males fed probiotic and prebiotic compared to synbiotic fed and control groups (P<0.05). The females fed prebiotic showed decrease in heart weight (P<0.05). The activity of ALP in females depressed by prebiotic consumption (P<0.05) whereas in males it was lower in probiotic fed group (P<0.05). AST activity in males fed prebiotic increased (P<0.05). In both genders CPK activity was higher in prebiotic and synbiotic fed groups (P<0.05). Results indicated using probiotic (*Saccharomyces cerevisiae*) has positive effects on performance and normal activity of enzymes. Prebiotic (*Thepax*) has positive effects on performance and reduction weight of heart and liver in Japanese quails. Synbiotic (*Saccharomyces cerevisiae* + *Thepax*) has positive effects on reduction weight of liver in both genders of Japanese quails.

Key words: Enzyme, Feed additive, Japanese quail, Performance

INTRODUCTION

Yeast and yeast products were being fed to farm animals for more than a hundred years (Owens and McCracken, 2007). It is well known that yeast culture, and its cell wall extract containing 1,3 - 1,6 D-glucan and Mannan oligosaccharide are the important natural growth promoters for modern livestock and poultry production (Van Leeuwen *et al.*, 2005 and Ghosh *et al.*, 2007). The advantages of these promoters over the traditional antibiotic growth promoters are: 1) no withdrawal time, 2) no residual effect, and 3) no causes of microbial mutation (Gibson and Roberfroid, 2008). Therefore, feeding yeast to broiler chicks improves body weight gain, feed conversion ratio (FCR) and survival rate (Owens and McCracken, 2007). Yeast (Thepax) acts as a probiotic in feed poultry (Fazli *et al.*, 2008). Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO 2002).Yeast stimulates the immunity system of the bird against pathogenic bacteria, especially *Salmonella, E. coli* and *Clostridiunz* (Ghadban, 2002) *and* reduces bird mortality chances (Kralik *et al.*, 2004). Yeast is also an excellent source of selenium and chromium; two trace minerals which may have positive effects of broiler health (Celik *et al*., 2001). It also acts as an antioxidant and its addition in broiler diet improves quality of broiler meat (Zhang *et al.*, 2005). Dietary live yeast as *Saccharomyces cerevisiae* (SC) 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). *Saccharomyces cerevisiae* is one of the effective adsorbent which is rich in crude protein (40-45%) with high biological value. It is also rich in vitamin B complex, biotin, niacin, pantathonic acid and thiamin (Reed and Nagodawithana, 1999). It is considered as one of the live microorganisms probiotic that, when administered through the digestive tract, have a positive impact on the hosts health through its direct nutritional effect (Banday and Risam, 2002). *Saccharomyces cerevisiae* contains enzymes alpha-amylase and protease that breakdown the starch and protein molecules, respectively, and help in digestion and efficient utilization. Cell wall of yeast *Saccharomyces cerevisiae* (*Thepax*) has known as prebiotic as is contain chitin, mannan and glucan that have been known as immune stimulant (Li and Gatlin, 2003 and Rodriguez *et al.,* 2003). Parlat *et al.* (2001) has reported that *Saccharomyces cerevisiae* yeast and its cell wall component (manner oligosaccharide) has known as minimizing the adverse effects of Aflatoxin (AF) in poultry on the basis of biological degradation. In present study, combination *Saccharomyces cerevisiae* and *Thepax* used as synbiotic. The synbiotic is the combined administration of specific prebiotic with probiotic to provide definite health benefits by synergistic action (Harish and Varghese, 2006). The effects of prebiotics, probiotics and synbiotics on serum enzymes (ALT, ALP, GGT, CPK, LDH and AST) had various reports. Hassaan *et al.* (2008) have reported that blood biochemical and hematological characteristics could be very important as indicator traits in breeding for the highest productive performance of birds. The goal of present study is investigate the effects of probiotic (*Saccharomyces cerevisiae*), prebiotic (*Thepax*) and synbiotic (combination of *Saccharomyces cerevisiae* and *Thepax*) on blood enzymes and performance values in male and female quails.

MATERIALS AND METHODS

Experimental design and housing: Present study was conducted at Islamic Azad University, Shabestar Branch-Iran in summer of 2010. A total of 192 one-day old Japanese quail chicks (mean body weight 7.78±0.39 gram) obtained from the Damavand quail Co. flock, were randomly assigned in 16 pens with 12 birds (6 males and 6 females) per pen and each bird occupied 0.015 m^2 of wiry floor space. The pens were randomized with respect to feed additives. Temperature was maintained at 35°C for the first 5 days and then gradually reduced according to normal management practices until a temperature of 22$^{\circ C}$ was achieved. Continuous lighting was maintained during the experimental period (2.5 watt/m^2).

Treatments and additives: The experiment was laid out under Completely Randomized Design (CRD), with four treatments and four replicates for each treatment. The nutrient composition of diets for quails during 1 to 42 days of age was based on the National Research Council (NRC, 1994) recommendations (Table 1). Treatment groups were: [1] basal diet without additive; [2] basal diet plus *Saccharomyces cerevisiae* at level of 2 g/kg; [3] basal diet plus *Thepax* at level of 1 g/kg; [4] basal diet plus combination of *Saccharomyces cerevisiae* at level of 1 g/kg + *Thepax* at level of 0.5 g/kg. Balanced diets were given *ad libitum* for all treatment groups from 1 to 42 days of age.

Table 1. Ingredient and calculated analysis of basal diet

Ingredients	Ration (%)
Yellow Corn	53.00
Soybean Meal, 44%CP	37.00
Fish Meal, 60%CP	5.50
Vegetable Oil	1.00
Oyster Shell	1.00
Mono Calcium Phosphate	1.50
DL-Methionine	0.15
Sodium Chloride	0.15
Mineral-Vitamin Premix*	0.50
Vitamin A	0.10
Vitamin E	0.10
Analysis results	
ME (Kcal/Kg)	2863
CP (%)	24.40
Calcium (%)	1.02
Available Phosphorus (%)	0.59
Methionine (%)	0.57
Methionine +cysteine	0.93
Lysine (%)	1.54

*Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; Biotin, 0.1 mg; Choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu,12 mg; I,1 mg; Se, 0.2 mg, and adequate antioxidant.

Figure1: Experimental room of Japanese quail, Tabriz-Iran (Instituted by Vahdatpour brothers)

Blood sampling and measurements: Feed intake (FI) of each experimental unit (each cage) was recorded. At the end of experimental period (42 d) the total body weight (BW) of birds in each cage was measured and then feed conversation ratio (FCR) was calculated. Before slaughtering the final BW of sample bird and after that weight of selected organs including liver and heart were recorded individually and presented as a percentage of live body weight. At 42 day of age, blood samples were collected in fasting state by cervical cutting of two birds (1 male and 1 female) per pen (n=8). Blood samples were rapidly centrifuged at 5000 rpm during 5 min and then sera by using commercial kits (Pars Azmun, Iran). Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Alanine transaminase (ALT), Gamma glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH) and Creatine phosphokinase (CPK) were assayed by auto analyzer (ALCYON 300) after international federation of clinical chemistry methods (IFCC, 2011). Then birds were processed by removing of the head, neck, shanks and feet and eviscerated by cutting around the vent then carefully removing the viscera. Carcass yield (dressing %) was obtained by expressing the dressed carcass weight as a percentage of live body weight.

Statistical analysis: The data were analyzed by an analysis of variance (ANOVA) technique using the General Linear Model (GLM) procedure of SAS (2001) and means were compared by Duncan`s Multiple Range test at P<0.05 level (Duncan, 1955).

RESULTS AND DISCUSSION

Performance values and mortality: The effects of three different feed additives on broiler's growth performance including Feed Intake (FI), Feed Conversion Ratio (FCR), Body Weight (BW) and dressing percentage are presented in Table 2. The results indicated that *Thepax* and SC had positive effects on performance of Japanese quails (P<0.05). Addition of *Thepax* (703±3) and SC (706±4) significantly increased FI compared to control group (697±7) (P<0.05). Other reports indicated that the addition of probiotics and organic acids to the broilers diet either numerically or significantly improved FI (Patterson and Burkholder, 2003). It was also observed that FCR in birds fed probiotic (3.04±0.11) and prebiotic (3.03±0.08) was higher (P<0.05) than control group (3.14±0.10) and birds fed synbiotic (3.15±0.07). Inborr (2000) reported that prebiotics (mainly oligosaccharides), probiotics and antibiotics markedly improved the general health status and FCR of the poultry. The findings of Soliman (2003) and Zhang *et al.* (2005) also supported the present results that supplementation of different levels of *saccharomyces cerevisiae* in boiler diets resulted in significant improvement in FCR. In the present study, birds fed probiotic and prebiotic demonstrated significantly higher BW (232±7 and 232±4gms, respectively) compared to control (222±6 gms) and synbiotic (222±4gms) groups (P<0.05). The significant improvement of BW and FCR could be attributed to the effect of probiotic and prebiotic which improve absorption of nutrients and depressed the harmful bacteria causing growth depression (El-Nagmy *et al.*, 2007). The results were also in accordance with Ghasemi *et al.* (2006) who used 0.10 and 0.20% *Saccharornyces cerevisiae* in broiler diet and found a significant increase in weight gain. In another study, Ahmadi (2001) reported that Probiotic (*saccrharomyces cerevisiae*) had significantly increased body weight gain in the birds fed diets supplemented with different levels of probiotic than the control group. Results indicated that consumption of probiotic (*Saccharomyces cerevisiae*) and prebiotic (*Thepax*) were more effective than synbiotic group in BW, FI and FCR of Japanese quails. The beneficial effects of the feed additives on quail`s performance parameters including FI, FCR, and BW are in agreement with previous studies (Kabir *et al*., 2004; Mountzouris *et al*., 2007 and Samli *et al*., 2007).

Table 2. Means growth performance values and mortality of quails fed additives up to 42 days of age.

Diets (treatments)	Feed Intake (g/bird)	Body Weight (g)	Feed Conversion Rratio (g/g)	Dressing (%)	Mortality (%)
Basal diet (control group)	697±7[b]	222±6[b]	3.14±0.10[b]	70.1±2[b]	Nil
Basal diet+SC	706±4[a]	232±7[a]	3.04±0.11[a]	74.9±2[a]	Nil
Basal diet+*Thepax*	703±3[a]	232±4[a]	3.03±0.08[a]	76.2±3[a]	Nil
Basal diet+SC+*Thepax*	700±3[ab]	222±4[b]	3.15±0.07[b]	73.7±1[a]	Nil

SC: Saccharomyces Cerevisiae. Means ± standard deviation (n=8)) within each column with no common superscript differ significantly (P<0.05)

Statistical analysis showed significantly improved (P<0.05) dressing percentages by feed additives compared to control group. Taksande *et al.* (2009) reported that average dressing percentage was highest in the treatment group fed with *Saccharomyces cerevisiae* compared to control and other probiotic treatment groups of Japanese quails. Good practical management during the experimental period resulted in no mortality (Table 2).

Heart and liver weights: The male birds fed each one of additives showed a significant decrease (P<0.05) in liver weight compared to control group. Whereas, the female birds fed synbiotic showed a significant decrease (P<0.05) in liver weight compared to control group (Table 3). These results are in agreement with previous studies (Azadegan Mehr *et al*., 2007; Babazadeh *et al*., 2011 and Vahdatpour *et al.,* 2011). Similarly, Mohan-Kumar and Christopher (1998) reported a significant decrease in liver relative weight due to lactobacillus and other beneficial microorganisms, present in probiotics; this might be attributed to prevention of pathogens from colonizing the gastrointestinal tract via competitive exclusion. With decrease in harmful microflora of intestine, less toxic byproducts will be produced, so that the liver would be under a less pressure for detoxifying these byproducts. Kaoud (2010) has reported that activation effect of the probiotic mixture *Lactobacillus sporogenes* (75×10^{8}c.f.u.), *Lactobacillus acidophilus* (330×10^{9} c.f.u.), *Saccharomyces cerevisiae* (125×10^{9} c.f.u.), alphaamylase 5 g and sea weed powder 100g) on the liver cells which improved the overall growth performance and reduce liver weight. The heart weight was significantly decreased (P<0.05) in the male birds fed probiotic and prebiotic compared to synbiotic fed and control groups. This is in agreement with Babazadeh *et al.,* (2011) who reported significantly reduction in the relative weight of heart in probiotic fed birds when compared with synbiotic fed and control groups. Perhaps one reason for lowering weight of heart can be depression negative effects of stressors on this organ (Vahdatpour *et al*., 2009). Ivanov (2004) reported more improvements in liver, gizzard and heart of broilers, mules and ducklings by supplementing diets with probiotics. The female birds fed prebiotic showed a significant decrease (P<0.05) in heart weight compared to other groups (Table 3).

Table 3. Means Heart and Liver weight (Percentage of live body weight) fed additives at 42 days of age.

Visceral organ (%)	Gender (Male or Female)	Basal diet (Control group)	Basal diet + SC	Basal diet + Thepax	Basal diet + SC+Thpax
Heart	M	0.98 ± 0.15^{a}	0.86 ± 0.15^{b}	0.88 ± 0.10^{b}	0.98 ± 0.09^{a}
	F	0.75 ± 0.04^{a}	0.75 ± 0.07^{a}	0.63 ± 0.09^{b}	0.71 ± 0.04^{a}
Liver	M	2.93 ± 0.42^{a}	2.18 ± 0.29^{b}	2.48 ± 0.53^{b}	1.82 ± 0.04^{c}
	F	2.73 ± 0.48^{a}	2.66 ± 0.45^{ab}	2.61 ± 0.75^{ab}	2.06 ± 0.22^{b}

SC: Saccharomyces Cerevisiae. Means±standard deviation (n=8) within each rows with no common superscript differ significantly (P<0.05).

Blood enzymes: Effects of dietary treatments on GGT, ALP, CPK, AST, ALT and LDH enzyme activities in male and female birds are summarized in Table 4. Dietary additives did not have any significant effect on activities of GGT and LDH enzymes. Male and female birds fed prebiotic showed a decrease in LDH activity compared to other groups. AST enzyme showed highest level of activity in prebiotic fed females birds as compared to other groups. A significant increase (P<0.05) in AST activity was noted in male birds fed prebiotics than that of control group. The male and female birds fed synbiotic demonstrated a highly significant increase (P<0.01) in CPK activity compared to control and probiotic groups. Effects of dietary additives on ALP activity showed a significant difference between male and female birds (P<0.05). Female birds fed prebiotic exhibited significantly low levels (P<0.05) of ALP activity compared to the control group. The results of ALP enzyme activity is in agreement with Vahdatpour *et al.* (2011). Similar results in male broiler chicks found by Mohamed and Mohamed (2009). Higher ALP and CPK activities in males than females could have been due to higher osseous (ALP) and muscular (CPK) development in males, just as it occurs in most vertebrates (Coppo, 2001). Effects of dietary additives on ALT activity showed a highly significant difference between male and female birds (P<0.05). The male birds fed probiotic showed a significant decrease in ALT activity (P<0.05) compared to control group. Significant differences in serum levels of ALP and ALT in male and female birds might be due to sexual differences in Japanese quails.

The current study indicated that feed additives in males caused increased AST levels and decreased ALT activity. Similar results were found by Babazadeh *et al.* (2011). On the other hand feed additives in females caused increased CPK and GGT activity and decreased ALP levels. SC+*Thepax* caused high levels of ALP, GGT, LDH, CPK and AST activities in serum of male quails. Imaeda (2000) indicated that increasing of enzyme utilized as indicators for clinical diagnosis of cardiac failure is in association with Sudden Death Syndrome (SDS). Other studies demonstrated that CPK, LDH, and AST activities were significantly increased in the serum of broilers chickens that died by SDS (Durdi and Aliakbarpour, 2005). The feed additives and especially SC+*Thepax* can be caused a circulatory enzymes elevation. These enzymes are not heart-specific, for definitive diagnosis we should account the other laboratory data and clinical observations carefully. Ozyurt *et al.* (2006) reported that AST, ALT, GGT and LDH usually appear in serum when damage is on the liver and muscle tissues caused by excessive stress. This study demonstrated that SC caused low ALP, GGT and CPK activities in serum of male birds compared to SC+*Thepax* and *Thepax*®, and it caused a significant decrease (P<0.05) in serum level of ALT in male birds compared to control group. Furthermore, SC showed a significantly decrease in live body weight of liver and heart of male quails compared to control and SC+*Thepax* groups. Ahmadi (2001) indicated that probiotic treatment groups decreased live body weight of heart and liver compared to control group. It can be concluded that SC by decreasing effects of stress can be caused a lower enzyme activity, lower heart and liver weight and it can be a protective agent for liver and muscles against damage factors in male quails compared to other additives fed groups. Lai *et al.* (2008) reported that pretreatment by adding *Saccharomyces cerevisiae* (0.5-2.0 g/kg) can reduce liver injury indexes and levels of AST and ALT enzymes. In addition to, consumption feed additives caused to decreasing of ALT activity in male birds compared to control group and it can help to health of liver and muscles as a protector agent.

Table 4. Means blood enzymes of quails fed additives at 42 days of age

Basal diet		(control group)	Basal diet+ SC	Basal diet+ Thepax	Basal diet+ SC+Thepax
M	AST	234.00[b]	273.00[ab]	291.50[a]	275.75[ab]
	ALT	24.75[a]	16.25[b]	22.75[ab]	20.50[ab]
	LDH	632.80	673.50	554.80	806.80
	CPK	658.50[ab]	635.30[b]	938.80[ab]	999.30[a]
	GGT	7.00	5.75	7.75	9.50
	ALP	1314.30	1294.50	1410.80	1669.30
	AST	274.50	273.25	302.00	279.50
	ALT	32.50	23.25	33.00	28.75
F	LDH	637.25	707.50	530.75	701.50
	CPK	687.50[b]	816.30[ab]	982.30[ab]	1200.00[a]
	GGT	5.25	11.50	6.25	10.25
	ALP	2315.00[a]	1698.00[ab]	1459.30[b]	1592.30[ab]

SC: Saccharomyces Cerevisiae. Means±standard deviation (n=8) within each rows with no common superscript differ significantly ($P<0.05$).

Conclusion Consumption of *Saccharomyces cerevisiae* or *Thepax* was more effective in improving performance and growth of Japanese quail. Especially, males and females fed *Thepax* have important factors for animal health. Intake of *Saccharomyces cerevisiae* caused a lower enzyme activity, decreasing liver weight and reducing effects of stress in male Japanese quails. Thereby it can be a protective agent for liver and muscles against damage factors in male quails compared to other feed additives.

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