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Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (*Coturnix japonica*)

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ABSTRACT

The present study was conducted to evaluate the influence of probiotic, prebiotic and synbiotic on performance values and blood enzymes of Japanese quails. One-day-old Japanese quail chicks (192), mean body weight 7.78 g, were randomly assigned in 4 treatments and 4 replicates. The experimental diets consisted of a basal diet without additive (control), 0.2 g/kg probiotic (P), 1.6 g/kg prebiotic (F) and 1.0 g/kg synbiotic (B) added to the basal diet.

Birds fed synbiotic showed a significant elevation in body weight compared to other groups. Feed intake of birds fed synbiotic and prebiotic were higher than control and probiotic fed groups. Birds fed synbiotic exhibited a better feed conversion ratio (3.08) compared to probiotic fed (3.19) and control groups (3.14).

The males fed additives showed decrease in liver weight. Females fed prebiotic showed decrease in liver weight compared to control and synbiotic additive groups. The relative weight of heart was decreased in the males fed prebiotic compared to synbiotic fed and control groups. The females fed probiotic showed increase in heart weight. The activity of ALP in males elevated by synbiotic consumption. ALP activity decreased in females fed additives. ALT activity was depressed in males fed probiotic or synbiotic. AST activity in males fed prebiotic elevated. In both gender CPK activity was higher in prebiotic feeding group.

Results indicated that using synbiotics has positive effects on performance and normal activity of enzymes and prebiotic has positive effects on performance and reduction weigh of heart and liver in Japanese quails.

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

The continued feeding of antibiotics at sub-therapeutic levels to poultry has created concerns about the extent to which usage increases the possibilities of antibiotic residue, the development of drug-resistant bacteria, and a reduction in the ability to cure these bacterial diseases in humans (Donoghue Dan 2003). Increased awareness of the potential problems associated with the use of antibiotics has stimulated research efforts to identify alternatives to their use as feed additives. In present study a probiotic, a prebiotic and a synbiotic were chosen as feed additives.

Probiotic, live microorganisms, confer a health benefit on the host (FAO/WHO 2002). Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995). The prebiotic approach does not have a long history of use in broiler chickens (Yang *et al*. 2009).

The goal of present study is indicating the effects of commercially procured probiotic, prebiotic and synbiotic on serum enzymes and growth performance values in male and female quails.

MATERIALS AND METHODS

Experimental design and housing: Day-old Japanese quail chicks (192), mean body weight 7.78±0.39 g, that 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°C was achieved. Continuous lighting was maintained in all experimental period (2.5 watt/m^2).

Treatments and additives: The experimental design was a completely randomized design (CRD), with 4 treatments and 4 replicates for each treatment. Nutrients compositions of diets for quails at 1 to 42 days old were based on the National Research Council (NRC 1994) recommendations

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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)	2863.00
CP (%)	24.40
Calcium (%)	1.02
Available phosphorus (%)	0.59
Methionine (%)	0.57
Methionine +cyctine	0.93
Lysine (%)	1.54

*Supplemented for kg of the diets: Vit. A, 12000 IU; D^3, 2000 IU; E, 20 mg; K^3, 3 mg; B^2, 7 mg; B^3, 12 mg; B^5, 3 mg; B^{12}, 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.

(Table 1). Treatment groups followed of: (1) basal diet without additive; (2) basal diet plus a multi-strain probiotic in dry white powder form (2×10^9 cfu/g: containing *Streptococcus salivarius* sub sp. *Thermophilus, Lactobacillus (L) delbruckii* sub sp. *bulgaricus, L. acidophilus, L. plantarum, L. rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, Candida pintoloppesii, and Asperigillus oryzae*) at level of 0.2 g/kg; (3) basal diet plus a prebiotic (*Aspergillus meal*) at level of 1.6 g/kg; (4) basal diet plus a synbiotic (a combination of *Enterococcus faecium* as probiotic strain, oligosaccharides as prebiotic, phytogenic substances and cell wall fragments) at level of 1 g/kg. Balanced diets were given *ad lib.* for all treatments at 1 to 42 days old.

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's of age in fasting state, blood samples were collected by cervical cutting of 2 birds (1 male and 1 female) per pen (N,8) and rapidly were centrifuged at 5000 rpm during 5 min and then sera was analysed by using commercial kits for aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), gamma glutamyl transpeptidase (GGT), *lactate dehydrogenase* (LDH) and *creatine phosphokinase* (CPK) in auto analyzer (IFCC 2011).

Statistical analysis: The data of experiment were analyzed by an analysis of variance (ANOVA) 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).

RESULS AND DISCUTION

Studies showed some favourable responses for FI and FCR by dietary additives. Inborr (2000) reported that prebiotics (mainly oligosaccharides), probiotics and antibiotics markedly improved the general health status and FCR of the poultry. Similarly, some workers have reported increased growth and improved FCR as a consequence of fructo-oligosaccharide (FOS) inclusion in broiler diets (Fukata *et al*. 1999, Wut *et al*. 1999). The effects of 3 different types of feed additives that were added to control basal diet on growth performance values are presented in Table 2.

In present study, FI in birds fed synbiotic and birds fed prebiotic was higher (P<0.05) than birds of control group and birds fed probiotic. Present study indicates that prebiotic and synbiotic could improve feed intake compared to control group. Birds fed synbiotic additive have better FCR compared to probiotic additive and control groups (P<0.05). FCR was intermediate state for birds fed prebiotic. BW which is an important indicator of production and lighter birds on the average produce the lowest meat and egg mass, because this class would include mainly more unhealthy birds than the heavier birds (Singh and Nordskog 1982). In present study, BW in birds fed synbiotic was higher than probiotic fed and

Table 2. Means growth performance values and mortality of quails fed additives at 42 day of age

Diets (treatments)	Feed intake (g)	Body weight (g/g)	Feed conversion ratio (%)	Mortality (g/bird)
Basal diet (control group)	$697\pm7^{b*}$	222 ± 6^{bc}	3.14 ± 0.10^{a}	0.0
Basal diet + probiotic	701 ± 4^{b}	220 ± 7^{c}	3.19 ± 0.11^{a}	0.0
Basal diet + prebiotic	706 ± 3^{a}	226 ± 4^{ab}	3.12 ± 0.08^{ab}	0.0
Basal diet + synbiotic	706 ± 3^{a}	229 ± 4^{a}	3.08 ± 0.07^{b}	0.0

*Figures bearing different superscripts within a row differs significantly (P<0.05).

control groups (P<0.05). Moreover, birds fed prebiotic showed a significant increase (P<0.05) compared to birds fed probiotic group (P<0.05). Results indicated that consumption of synbiotic and then prebiotic were more effective than other groups in BW, FI and FCR of Japanese quail.

The present study demonstrated that the synbiotic and prebiotic products displayed a greater growth-promoting effect than the probiotic and control groups. The beneficial effects of the synbiotic and prebiotic products 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). The results of present study about the effects of probiotic in BW, FCR and FI are in agreement with the study of Maiolino *et al.* (1992) which reported that probiotics did not have any significant positive effect on broilers. Barrow (1992) indicated that there was little evidence in the studies to support the claims of positive effects made by probiotics and the studies may have suffered from errors in methodology and interpretation. Several studies showed that addition prebiotic to poultry diets enhanced performance (Grimes *et al.* 1997 and Rodriguez *et al.* 2005, Navidshad *et al.* 2010).

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 prebiotic showed a significant decrease (P<0.05) in liver weight compared to birds fed synbiotic and control groups (Table 3). These results are in agreement with the study of Azadegan Mehr *et al*. (2007). Similarly, Mohan-Kumar and Christopher (1988) reported a significant decrease in liver relative weight due to lactobacillus and other beneficial microorganisms, which are present in probiotics, can prevent 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. The heart weight significantly decreased (P<0.05) in the male birds fed prebiotic-additive compared to synbiotic and control groups. The female birds fed probiotic showed a significant increase (P<0.05) in heart weight compared to other groups (Table 3). Increasing heart weight in female birds fed probiotic probably could be cause to heart hypertension or appearing to ascites disease. Good management of present experiment caused no disease and mortality (Table 3).

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. GGT enzyme activity showed the lowest level of activity in males fed probiotic compared to other groups and it showed the lowest level of activity in females of control group

Table 3. The effect of dietary treatments on heart and liver weights (g) of quails at 42 days of age

Diets (treatments)	Heart		Liver	
	Male	Female	Male	Female
	(Percentage of live weight)			
Basal diet (control group)	0.98±0.15^{a*}	0.75±0.04^{b}	2.93±0.42^{a}	2.73±0.49^{a}
Basal diet + probiotic	0.89±0.04^{ab}	0.90±0.05^{a}	1.88±0.38^{b}	2.55±0.42^{ab}
Basal diet + prebiotic	0.81±0.00^{b}	0.71±0.07^{b}	1.98±0.09^{b}	2.31±0.26^{b}
Basal diet + synbiotic	0.92±0.07^{a}	0.71±0.06^{b}	1.70±0.14^{b}	2.81±0.46^{a}

*Figures bearing different superscripts within a row differ significantly (P<0.05).

Table 4. Effects of feed additives on blood enzymes of Japanese quails at 42 days of age

	Male				female			
Control	Probiotic	Prebiotic	Synbiotic	Enzymes	Control	probiotic	Prebiotic	Synbiotic
GGT	7.00±0.35^{a}	6.50±0.26^{a}	9.00±0.68^{a}	8.50±0.55^{a}	5.25±0.15^{a}	10.25±0.67^{a}	12.25±0.61^{a}	7.75±0.42^{a}
ALP	1314.25 ±14.69^{b}	1513.50 ±19.76^{b}	1335.75 ±12.66^{b}	1816.75 ±25.36^{a}	2315.00 ±26.30^{a}	1713.25 ±33.44^{b}	1462.75 ±30.70^{b}	1600.75 ±18.28^{b}
CPK	658.50 ±27.26^{b}	663.50 ±2.14^{b}	1145.50 ±7.76^{a}	844.50 ±23.36^{b}	687.50 ±25.29^{b}	728.00 ±7.00^{ab}	1030.00 ±16.78^{a}	798.00 ±21.05^{ab}
AST	234.00 ±4.50^{b}	259.50 ±3.28^{ab}	292.25 ±3.49^{a}	267.50 ±2.52^{ab}	274.50 ±2.69^{a}	284.50 ±2.82^{a}	278.75 ±4.38^{a}	283.00 ±2.84^{a}
ALT	24.75 ±0.34^{a}	18.00 ±0.35^{b}	20.02 ±0.38^{ab}	17.50 ±0.21^{b}	32.50 ±0.87^{a}	29.00 ±1.62^{a}	25.50 ±80^{a}	26.75 ±1.24^{a}
LDH	632.75 ±12.97^{a}	637.75 ±20.43^{a}	841.50 ±14.39^{a}	653.25 ±28.88^{a}	637.25 ±0.84^{a}	606.75 ±11.98^{a}	673.25 ±18.80^{a}	616.75 ±18.02

*Figures bearing different superscripts within a row differs significantly (P<0.05).

compared to other groups. LDH enzyme showed lowest level of activity in females fed probiotic compared to other groups and it exhibited the lowest level of activity in males of control group compared with other group. Effects of dietary additives on ALP activity showed a highly significant difference between male and female birds ($P<0.01$). The female birds fed additives exhibited significantly low levels ($P<0.05$) of ALP activity compared to the control group ($P<0.05$). The biochemical analysis showed a significant increase ($P<0.05$) in activity of ALP in male birds fed synbiotic compared to the other groups. Similar results were reported by Hashem and Mohamed (2009). Significant differences in serum levels of ALP in male and female birds can be result of sexual differences in Japanese quails. Higher ALP and CPK activities in males than females could have been due to higher osseous (ALP) and muscular (CPK) male development, just as it occurs in most vertebrates (Coppo 2001). Effects of dietary additives on ALT activity showed a significant difference between male and female birds ($P<0.05$). The male birds fed probiotic or synbiotic showed a significant decrease in ALT activity ($P<0.05$) compared to control group. In female birds, additive fed groups exhibited low levels of ALT activity compared to control group. Male and female birds fed additives showed increased AST activity compared to control group. The male birds fed prebiotic showed a significant increase ($P<0.05$) in AST activity compared to control group. However, the female birds fed probiotic showed an increase in serum levels of AST compared to other groups but it was not a significant increase. The male birds fed prebiotic showed significantly ($P<0.05$) high levels of CPK activity compared to other groups. Whereas, increase in CPK activity in other groups did not have a significant difference compared to control group. The female birds fed prebiotic showed a significant difference ($P<0.05$) in serum levels of CPK activity compared to control group.

Present study indicated that feed additives caused to higher levels of AST and CPK activities and prebiotic caused the high levels of GGT and LDH activities in serum of male and female birds compared to control group. In an investigation Imaeda (2000) indicated that increase serum level of enzyme utilized as indicators for clinical diagnosis of cardiac failure is in association with sudden death syndrome (SDS). Studies demonstrated that CPK, LDH, and AST activities significantly increased in the serum of broilers chickens that died by SDS (Imaeda 1999, Durdi and Aliakbarpour 2005). The feed additives and especially prebiotic can be caused a circulatory enzymes elevation. Since 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 there is damage on the liver and muscle tissues caused by excessive stress. Cyber (1999) and Panda *et al*. (2000) reported that probiotic has been promoted to reduce stress. This study demonstrated that probiotic caused low AST, GGT and LDH activities in serum of male birds compared to synbiotic and prebiotic, and it caused significant decrease ($P<0.05$) in serum level of ALT in male birds compared to control group. It can be concluded that probiotic by decreasing effects of stress can cause a lower enzyme activity and it can be a protective agent for liver and muscles against damage factors in male quails compared to groups fed other additives. Furthermore, consumption of all feed additives decreased ALT activity in male and female birds compared to control group and they can help in health of liver and muscles as a protector agent.

The present study demonstrated that the synbiotic and prebiotic displayed a greater growth-promoting effect than the probiotic, and the control group. Synbiotic seemed more effective in performance of Japanese quail than other additives. Consumption of prebiotic in males and females reduced in heart weight that is important factor for animal health. Probiotic intake can cause a lower enzyme activity and decreasing effects of stress. Thereby it can be a protective agent for liver and muscles against damage factors in male quails compared to other feed additives. In addition to diagnosing enzymatic activity, and of heart weight enhancing in female Japanese quail fed probiotic will be stimulating for more studies.

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