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Effects of Protexin®, Fermacto® and combination of them on blood enzymes and performance of Japanese quails (*Coturnix Japonica*)

Tohid Vahdatpour[1*], Hossein Nikpiran[2], Daryoush Babazadeh[3], Sina Vahdatpour[3] and Mohammad Ali Jafargholipour[3]

[1]Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran
[2]Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[3]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

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ABSTRACT

The aim of present study was to evaluate the influence of probiotic, prebiotic and synbiotic on performance values and blood enzymes of Japanese Quails. Total of 192 one-day old Japanese quail chicks 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 (Protexin®), 1.6 g/kg prebiotic (Fermacto®) and 1.0 g/kg synbiotic (Protexin®+ Fermacto®) added to the basal diet. Birds fed synbiotic elevated body weight compared to other groups (P<0.05). Feed intake of birds fed synbiotic and prebiotic were higher than control and probiotic fed groups (P<0.05). Birds fed synbiotic exhibited a better feed conversion ratio (3.09) compared to probiotic fed (3.19) and control groups (3.14) (P<0.05). The males fed additives showed decrease in liver weight (P<0.05). Females fed prebiotic and synbiotic showed decrease in liver weight compared to control group (P<0.05). The relative weight of heart was decreased in the males fed prebiotic and synbiotic compared to control group (P<0.05). The females fed probiotic showed increase in heart weight (P<0.05). The activity of ALP in females depressed by prebiotic consumption (P<0.05). In males fed probiotic or synbiotic ALT activity was depressed (P<0.05). LDH activity in males fed synbiotic depressed (P<0.05). In both gender CPK activity was higher in prebiotic and synbiotic feeding groups (P<0.05). Results indicated that using synbiotics (Protexin®+ Fermacto®) has positive effects on performance and normal activity of enzymes. Prebiotic (Fermacto®) has positive effects on performance and reduction weigh of heart and liver in Japanese quails.

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

Abbreviations: *FI, feed intake; BW, body weight; FCR, feed conversion ratio; AST, Aspartate aminotransferase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma glutamyl transpeptidase; LDH, Lactate dehydrogenase; CPK, Creatine phosphokinase; SDS, sudden death syndrome.*

INTRODUCTION

Historically, there has been widespread use of antibiotics in animal feed for improving growth rate and feed efficiency, as well as for the prevention and treatment of diseases. However, the continued feeding of antibiotics at sub-therapeutic levels has created concerns about the extent to which usage increases the possibilities of antibiotic residues in the carcass of the birds, the development of drug-resistant bacteria [41], and a reduction in the ability to cure these bacterial diseases in humans [7]. 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. The use of feed additives has 2 objectives: (I) the control of pathogen microorganisms and (II) to enhance the digestive microflora with beneficial microorganism [39]. In present study a probiotic (Protexin®), a prebiotic (Fermacto®) and a synbiotic (combination of Protexin® and Fermacto®) were chosen as feed additives.

1.1. Probiotic

Probiotic are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [11]. The beneficial modes of action of probiotics include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, expression of bacteriocins [24], enzymatic activity inducing absorption and nutrition [42], immune modulatory effects [35], inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa [17]. The probiotic of this study was Protexin® [33]. Protexin® is a multi-strain probiotic used in poultry feed [1]. It contains naturally occurring nine different species of beneficial microflora which are generally regarded as safe by the American food and drug administration [15]. Protexin® is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts *(Lactobacillus plantarum* 1.89×10^{10} cfu/kg,*Lactobacillus delbrueckii* subsp. *Bulgaricus* 3.09×10^{10} cfu/kg, *Lactobacillus acidophilus* 3.09×10^{10} cfu/kg, *Lactobacillus rhamnosus* 3.09×10^{10} cfu/kg, *Bifidobacterium bifidum* 3.00×10^{10} cfu/kg, *Streptococcus salivarius* subsp. *Thermophilus* 6.15×10^{10} cfu/kg, *Enterococcus faecium* 8.85×10^{10} cfu/kg, *Aspergillus oryza* 7.98×10^{9} cfu/kg, *Candida pintolopesii* 7.98×10^{9} cfu/kg) [1].

1.2. Prebiotic

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" [16]. The prebiotic approach has not a long history of use in broiler chickens [44]. However, application studies have been increasing in the last years to assess their effect on gut health, performance, and reduction of pathogen shedding. The prebiotic of this study is Fermacto® [13]. The commercially available fermentation product referred to as *Aspergillus Meal* (AM), has no live cells or spores and is proven to enhance the digestive efficiency of the gut [18].

1.3. Synbiotic

Synbiotics may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract [16]. The results on *in vivo* trials are promising, showing a synergistic effect coupling probiotics and prebiotics in the reduction of food-borne pathogenic bacterial populations [5]. The synbiotic of present study was combination of Protexin® and Fermacto®.

The effects of prebiotics, probiotics and synbiotics on serum enzymes (ALT, ALP, GGT, CPK, LDH and AST) and growth performance of poultry particularly had various reports. Inborr [22] has reported that prebiotics (mainly oligosaccharides) and probiotics markedly improved the general health statues and feed efficiency of the poultry. Vahdatpour et al. [43] and Hassaan et al. [19] 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 normal concentration of serum enzymes would be change by abnormal factors. Serum AST, ALT, ALP, GGT activities (liver enzymes) were used to evaluated liver function, the increase in their activities are related to degenerations of hepatocytes or liver damage irrespective of its origin [12]. Scholl et al. [38] has 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. In broiler chickens the CPK is released into the circulation in response to various pathological and exposure to environmental stressors [4]. However, there are different reasons for changing the concentration and the activity of blood enzymes. Many enzymes register important physiological variations due to age, sex, genital stage, diet, physical exercise and other variables [6]. The goal of present study is indicating the effects of probiotic (Protexin®), prebiotic (Fermacto®) and synbiotic (combination of Protexin® and Fermacto®) on serum enzymes and growth performance values in male and female quails.

MATERIALS AND METHODS

2.1. 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 that were provided from the Damavand quail Co. flock and randomly assigned in 16 pens with 12 birds (6 males and 6 females) per each 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).

2.2. Treatments and additives

The experimental design was Completely Randomized Design (CRD), with four treatments and four replicates for each treatment. Nutrients compositions of diets for quails at 1 to 42 days old were based on the National Research Council [28] recommendations (Table 1). Treatment groups followed of: 1] basal diet without additive; 2] basal diet plus Protexin® (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 Fermacto® (*Aspergillus meal*) at level of 1.6 g/kg; 4] basal diet plus combination of Protexin® at level of 0.1 g/kg and Fermacto® at level of 0.8 g/kg. Balanced diets were given *ad libitum* for all treatments at 1 to 42 days old.

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

Figure1: Experimental room of Japanese quail, Tabriz-Iran

**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.*

2.3. 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 calculation. 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 in fasting state, bloods samples were collected by cervical cutting of two birds (1 male and 1 female) per pen (N=8) and rapidly were centrifuged at 5000 rpm during 5 min and then sera by using commercial kits (Pars Azmun, Iran) for AST, ALP, ALT, GGT, LDH and CPK in auto analyzer (ALCYON 300) were assayed by international federation of clinical chemistry methods [20].

2.4. Statistical analysis

The data of experiment were analyzed by an analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS [37] and means were compared by Duncan`s multiple Range test at $P<0.05$ level [8].

RESULTS AND DISCUTION

3.1. Performance values and mortality

The primary role of a diet is not only to provide enough nutrients to fulfill metabolic requirements of the body but also to modulate various functions of the body. Probiotics, prebiotics, and synbiotics are either beneficial microorganisms or substances that facilitate the growth of these microorganisms, which can be suitably harnessed by the food manufacturers and hold considerable promise for the health care industry. Studies have also shown some favorable responses for FI and FCR by dietary additives. Inborr [22] has 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 [14]. The effects of three different types of feed additives that were added to control basal diet on growth performance values are presented in Table 2. FI in birds fed synbiotic (708±3 g/bird) and birds fed prebiotic (706±3 g/bird) was higher ($P<0.05$) than birds of control group (697±7 g/bird) and birds fed probiotic (701±4 g/bird). On the other hand 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 [40]. In present study, BW in birds fed synbiotic (229±2 g) was higher than other groups which had a significant increase ($P<0.05$) compared to probiotic group. The significant improvement of BW and FCR could be attributed to the effect of probiotic and prebiotic which improve absorption of nutrients and depressed harmful bacteria that cause growth depression [10]. Results indicated that consumption of synbiotic (Protexin®+ Fermacto®) and then prebiotic (Fermacto®) were more effective than other groups in BW, FI and FCR of Japanese quails. Good management of present experiment cause to no faced to any disease and mortality (Table 2).

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

Diets (treatments)	Feed intake (g/bird)	Body Weight (g)	Feed Conversion Ratio (g/g)	Mortality (%)
Basal diet (Control group)	$697\pm7^{b*}$	222 ± 6^{bc}	3.14 ± 0.10^{a}	0.0
Basal diet + Probiotic (Protexin®)	701 ± 4^{b}	220 ± 7^{c}	3.19 ± 0.11^{a}	0.0
Basal diet + Prebiotic (Fermacto®)	706 ± 3^{a}	226 ± 4^{ab}	3.12 ± 0.08^{ab}	0.0
Basal diet + Synbiotic (Protexin®+ Fermacto®)	708 ± 4^{a}	229 ± 3^{a}	3.09 ± 0.06^{b}	0.0

*****differ significantly ($P<0.05$).*

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 [27, 36]. 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. [23] which reported that probiotics did not any significantly positive effect on broilers. A critical appraisal of some studies conducted in the 1970's and early 1980 are on the effectiveness of probiotics in broilers and layers. Barrow [23] 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 have shown that addition Fermacto® to poultry diets enhanced performance [29, 34].

3.2. 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 prebiotic or synbiotic showed a significant decrease (P<0.05) in liver weight compared to control group (Table 3). These results are in agreement with the study of Azadegan Mehr et al. [2]. Similarly, Mohan-Kumar and Christopher [26] 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 was significantly decreased (P<0.05) in the male birds fed prebiotic and synbiotic compared to control group. The female birds fed probiotic showed a significant increase (P<0.05) in heart weight compared to prebiotic and control groups (Table 3). Increasing heart weight in female birds fed Protexin® probably could be cause to heart hypertension or appearing to ascites disease.

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 (Protexin)	0.89±0.04^{ab}	0.90±0.05^{a}	1.88±0.38^{b}	2.55±0.42^{ab}
Basal diet + Prebiotic (Fermacto)	0.81±0.00^{b}	0.71±0.07^{b}	1.98±0.09^{b}	2.31±0.26^{b}
Basal diet + Synbiotic (Protexin+ Fermacto)	0.81±0.04^{b}	0.83±0.06^{ab}	1.97±0.12^{b}	2.41±0.28^{b}

*****differ significantly (P<0.05).

3.3. 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 AST enzymes. Numerically, the lowest GGT enzyme activity showed in female birds of control group and in male birds of probiotic group compared to other groups. Male and female birds fed additives showed an increase in AST activity compared to control group. LDH enzyme showed lowest level of activity in females fed probiotic compared to other groups and in male birds fed synbiotic a significant decrease (P<0.05) in LDH activity was occurred compared to prebiotic group. The male and female birds fed synbiotic and prebiotic 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). The biochemical analysis showed an increase in ALP activity of male birds and a decrease in ALP activity of female birds which influenced by additive consumption. Female birds fed prebiotic exhibited significantly low levels (P<0.05) of ALP activity compared to the control group. Similar results in male broiler chicks found by Mohamed and Mohamed [25]. 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 [6].

Effects of dietary additives on ALT activity showed a highly significant difference between male and female birds (P<0.01). The biochemical analysis showed a decrease in ALT activity of male and female birds fed additives compared to control groups. The male birds fed probiotic or synbiotic 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 can be resulted of sexual differences in Japanese quails.

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

	Male				female			
Enzymes	Control	probiotic	prebiotic	synbiotic	control	probiotic	prebiotic	synbiotic
GGT	7.00±0.35^{a}	6.50±0.26^{a}	9.00±0.68^{a}	10.50±0.44^{a}	5.25±0.15^{a}	10.25±0.67^{a}	12.25±0.61^{a}	10.00±0.51^{a}
ALP	1314.30±14.69^{a}	1513.50±19.76^{a}	1335.80±12.66^{a}	1676.80±14.56^{a}	2315.00±26.30^{a}	1713.30±33.44^{ab}	1462.80±30.70^{b}	1804.50±18.^{ab}
CPK	658.50±27.26^{b}	663.50±2.14^{b}	1145.50±7.76^{a}	1117.50±14.53^{a}	687.50±25.29^{b}	728.00±7.00^{b}	1030.00±16.78^{a}	1187.50±18.5^{a}
AST	234.00±4.50^{a}	259.50±3.28^{a}	292.25±3.49^{a}	266.50±3.12^{a}	274.50±2.69^{a}	284.50±2.82^{a}	278.75±4.38^{a}	279.75±3.21^{a}
ALT	24.75±0.34^{a}	18.00±0.35^{b}	20.00±0.38^{ab}	16.75±0.25^{b}	32.50±0.87^{a}	29.00±1.62^{a}	25.50±80^{a}	27±1.10^{a}
LDH	632.80±12.97^{ab}	637.8±20.43^{ab}	841.50±14.39^{a}	485.30±16.38^{b}	637.25±0.84^{a}	606.80±11.98^{a}	673.30±18.80^{a}	658.80±17.04^{a}

*differ significantly (P<0.05).

Present study indicated that feed additives can cause high levels of AST, GGT and CPK activities in serum of male and female quails. Fermacto® caused to elevation LDH activity in serum of male and female birds compared to other groups. In an investigation, Imaeda [21] 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 were significantly increased in the serum of broilers chickens that died by SDS [9]. The feed additives and especially Fermacto® 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. [30] 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. Panda et al. [31] reported that Protexin® has been promoted to reduce stress. This study demonstrated that Protexin® caused low AST, GGT and CPK activities in serum of male birds compared to Protexin®+Fermacto® and Fermacto®, and it caused a significant decrease (P<0.05) in serum level of ALT in male birds compared to control group. It can be concluded that Protexin® by decreasing effects of stress can be caused a lower enzyme activity and it can be a protective agent for liver and muscles against damage factors in male quails compared to other additives fed groups. Furthermore, consumption of all feed additives caused to decreasing of ALT activity in male and female birds compared to control group and they can help to health of liver and muscles as a protector agent.

CONCLUSION

The present study demonstrated that the Fermacto® and the combination of Protexin® and Fermacto® displayed a greater growth-promoting effect than the Protexin® and the control

group. Protexin®+ Fermacto® seemed more effective in performance of Japanese quail than other additives. Consumption of Fermacto® in males and females caused important factors for animal health by reducing heart weight. Protexin® intake can be caused 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, finding the main reason of heart weight enhancing in female Japanese quail fed Protexin® will be important for more studies.

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