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Effects of Functional Feed Additives on Growth Influenced Hormones and Performance of Japanese Quails (*Coturnix japonica*)

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ABSTRACT

The objective of this research was to determine the effects of Protexin®, Fermacto® and its compounded effects on growth performance, visceral organs weight, insulin, thyroxin and growth hormone of Japanese quails. Growth performance with respect to body weight gain and visceral organs weight were recorded and then blood sampling were taken for analysis of serum hormones using ELISA technique. The analyzing of data in completely randomized design showed that consumption of each Fermacto® and Protexin®+Fermacto® caused elevation in feed intake and body weight ($p<0.05$). The gizzard weight in males fed Fermacto® was decreased and in females fed all additives, especially protexin® was elevated ($p<0.05$). Males and Females fed Fermacto® have lighter intestines weight than other additive groups ($p<0.05$). The heart weight in males fed additives were reduced and in females fed Protexin® were elevated ($p<0.05$). In males fed additives liver weight was lighter than control group ($p<0.05$). In females fed Fermacto® and Protexin® + Fermacto® liver weight was reduced ($p<0.05$). Insulin level increased ($p<0.05$) by feeding of Fermacto and Protexin®+ Fermacto® in female and males respectively. The level of thyroxin in females depressed by feed additive consumption ($p<0.05$). Growth hormone level was elevated by consuming of feed additives in female birds ($p<0.05$). It was concluded that consumption of each Fermacto® and Protexin®+ Fermacto® in quail's diet can improve growth performance values by increasing the feed intake and stimulating the insulin and growth hormone release.

INTRODUCTION

Currently, Japanese quail had gained attention in poultry industry as they are resistant to pathogens and a good producer of organic egg and meat for healthy nutrition in human and is being used as beneficial animal model in researches (Bishop, 2009). The global paradigm is shifting from an emphasis on productive efficiency to one of public concerns (Cakir *et al*., 2008), as long term use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria, accumulation of drug residues in the body of the birds, imbalance of normal microflora and finally, harmful effects on human health (Attia *et al*., 2011). Therefore, in the poultry industry, the application of antibiotics as growth promoters and prevention of the poultry diseases by competing with the pathogens are gradually being lost. Challenges for finding safe alternatives have been started and continued. The use of feed additives has 2 objectives: (I) the control of pathogenic microorganisms and (II) to enhance the digestive microflora with beneficial microorganism. Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). The beneficial modes of action of probiotics include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, expression of bacteriocins (Mazmanian *et al*., 2008), enzymatic activity inducing absorption and nutrition, immune response (Kaoud, 2010), inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa. A prebiotic was defined as non- digestible food ingredient that beneficially affects the host, selectively stimulating the growth or activity or both of one or a limited number of bacteria present in intestine. This approach which seems to be the best efficacy of use in these products is synbiotic, which is a combination of both probiotics and prebiotics and which synergistically affects the host by improving the survival and implantation of live microbial dietary additives and their necessary nutrients and suitable environment in the gastrointestinal tract (Attia *et al*., 2011). These feed additives promote gut health by several possible mechanisms including altering gut pH, maintaining protective gut mucus, selecting beneficial intestinal organisms acting against pathogens, enhancing fermentation acids, enhancing nutrient uptake and increasing the humoral immune response (Cakir *et al*., 2008). Finally, these metabolic changes in bird's physiology results in enhanced growth and performance of the host. Many factors are affected feed additive`s functions as survival and stability of the probiotic strain, specificity of the strain relative to host, dose and frequency of the additive administration, health and nutritional status of the host, type, gender, age, stress and genetics of the bird, condition of the feed being processed. Although, there are several hypotheses on the mode of action of feed additives on bird`s growth performance but these hypothesis are almost yet vague. Therefore, the present study was conducted to investigate the effects of Protexin® (probiotic), Fermacto® (prebiotic) and a mixture of Protexin® and Fermacto® (Protexin®+ Fermacto®) (synbiotic) on growth performance and three growth-related hormones in Japanese quail's for commercial production.

MATERIALS AND METHODS

Experimental design and housing

Present study was conducted at Islamic Azad University, Shabestar Branch-Iran in summer season. One hundred and ninety-two (192) newly hatched and healthy Japanese quail chicks (mean body weight 7.78 ± 0.39 g) were procured from the Damavand Quail Co. flock and kept in a specially designed farm in the city of Tabriz, Iran. The chicks were randomly placed in 4 treatment groups with 4 replicates per treatment and 12 chicks per replicate (48 birds/treatment) per wiry cages (0.3×0.5×0.35 m) and were established with a distance from one place to another at one room with controlled standard temperature, humidity, ventilation for quails housing. Temperature was maintained at 35˚C for the first 5 days and then gradually reduced according to normal management practices until the temperature reached 22˚C. Continuous lighting was maintained in all experimental period (2.5 watt/m^2) and kept for 42 days (Figure 1).

Dietary treatments

The basal diet (conventional mash) was formulated to meet and exceed the nutrient requirements of grower Japanese quails following National Research Council (NRC, 1994) (Table 1). Each feed additives in standard high level of manufactory recommendation was added to the basal diet and mixed completely and water were provided *ad libltum* during the experiment (1 to 42 days of age). The four treatment groups were as follow: 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.

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

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; 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.

Growth Measurements and blood sampling

Feed intake (FI) of each experimental unit (each cage) was recorded. At the end of feeding trial, one male and one female bird from each replicate (8 birds/treatment) were randomly selected, weighed and slaughtered by cervical cutting after 12h fast. Blood samples were collected without anti coagulant for serum separation. The gizzard, intestine, liver and heart were excised, weighed and calculated as a percentage of the live body weight and then recorded. The sera were separated by centrifugation at 3000 rpm for 8 min after 2 h incubation at room temperature and were analyzed by ELISA technique (AWAKNESS technology Inc., USA) including ELISA microplate reader (stat fax 2100) and automatic ELISA plate washer (state fax 2006) for growth related hormones estimation including, insulin (Biosource Inc, EASIA Kit. Belgium), growth hormone (Monobind Inc, ELISA Kit, USA) and thyroxin (T4 ELISA Kit, Pishtaz Teb, IRAN).

Statistical analysis

Analysis of variance (ANOVA) for completely randomized design (CRD) using the general linear model (GLM) procedure, Bartlett's test for homogeneity of data variance and standard division were calculated. Duncan's new multiple range test was used to compare the means of treatments at $P<0.05$ level (SAS Institute, 2001).

RESULTS AND DISCUSSION

Growth performance

The effects of three different types of feed additives that were added to basal diet on growth performance values are presented in Table 2. Consumption of all additives in basal diet of quails caused an elevation in FI compared to control group. FI in birds fed Protexin®+ Fermacto® (709±3 g/bird) and birds fed Fermacto® (706±3 g/bird) was higher (P<0.05) than birds of control group (697±7 g/bird) and birds fed Protexin® (701±4 g/bird). With increase of FI in each group fed by Fermacto® and Femacto®+ Protexin®, the BW showed elevation in comparison to control and Protexin® groups (p<0.05). The FC in Fermacto® and Protexin®+ Fermacto® fed groups was better than Protexin® group (p<0.05). However, the birds fed Protexin showed an elevation in FC compared to other groups (p<0.05). By good management, in all period, groups faced no disease and mortality. The obtained results of present study confirmed the equal positives effects of both Fermacto® as prebiotic and Fermacto®+ Protexin® as synbiotic and depressed effects of Protexin® as probiotic on performance values. In present study, the beneficial effects of prebiotic and synbiotic on Japanese quail performance parameters including BW, FI and FC are in agreement with previous studies (Vahdatpour *et al.,* 20 1 1) . Researchers have previously demonstrated significant increases in body weight gain in broilers receiving diets supplemented with probiotics and prebiotics (Babazadeh *et al.,* 2011). El-Husseiny *et al.* (2008) reported that chicks fed diets containing diamond VXPC, 0.1 % as prebiotic significantly recorded the highest BW and best value of FC in comparison to negative control group (p<0.05). Saleh *et al.* (2006) concluded that tomoko (dried aspergillus) as prebiotic is effective to improve the performance of broiler. The significant improvement of BW and FC could be attributed to the effect of probiotic and prebiotic which improve absorption of nutrients and depressed harmful bacteria that cause growth depression (EL-Nagmy *et al.,* 2007). Similar findings were reported by Mateova *et al.* (2008) who confirmed the favorable effect of probiotics, prebiotics and their patenting effect on growth and state of health of broiler chickens. Khosravi *et al.* (2008) found that using Protexin in broiler diets had no significant effect on BW, FI and FC at 42 days of age. Although, results show that the birds fed Protexin® had numerically higher BW than control diet. EL-Sheikh *et al.* (2009) reported that addition of MOS (0.2%) to basal diet of Mandarah chickens especially in males significantly increased BW and FI (p<0.05). Jouybari *et al.* (2009) reported that addition of pseudomonas putida and pantoea agglomeran (0.25%) as a new probiotic to basal diet of broiler chickens from 1-49 days of age significantly increased FI and improve FC (p<0.05). Several studies have shown that addition of Fermacto® to poultry diets enhanced performance (Navidshad, 2010). This good performance in birds fed by Fermacto® or Fermacto®+ Protexin® of present study may be related to improved intestinal lumen health and increased digestion and absorption of nutrients by different enzymes. Certainly, stimulation effects of palatability and texture of ration and reduction of pathogenic micro-organisms influenced by Fermacto and Fermacto+Protexin can be other hypothesis. However, addition of Protexin® as probiotic did not display an appropriated effect on performance values.

Table 2: Means growth performance values and mortality of quails fed additives at 42 days of age

Diets (treatments)	Feed intake (g/bird)	Body Weight (g)	Feed Conversion (g/g)
Basal diet (Control group)	697±7^{b}	222±6^{b}	3.14±0.10^{b}
Basal diet +Protexin	701±4^{b}	220±7^{b}	3.19±0.11^{a}
Basal diet +Fermacto	706±3^{a}	226±4^{a}	3.12±0.08^{b}
Basal diet +Protexin+fermacto	709±3^{a}	226±4^{a}	3.13±0.07^{b}

Means±standard deviation (n=8) within each column with no common superscript differ significantly (p<0.05).

Visceral organs weight

The effects of feed additives intake on visceral organs weight in male and female Japanese quail are presented in Table 3. The means of gizzard and intestines weights in males fed Fermacto® were decreased as compared to other groups (p<0.05). Males and females fed Protexin® have heavier gizzard and intestines compared to other groups. Intestines weight in female birds fed Protexin® were elevated as compared to other groups (p<0.05). Fermacto (3.01%) caused a significant decrease (p<0.05) in intestines weight of female birds as compared to Protexin® (4.83%) and Protexin®+ Fermacto® (3.68%). Gizzard weight in females fed all additives and especially Protexin® were significantly elevated as compared to control group (p<0.05). The means of heart weight in males fed additives were lighter and in females fed Protexin® were higher than control group (p<0.05). Additives intake in male and females caused decrease in liver weight. Liver weight in females fed Protexin®+ Fermacto® (2.41%) and Fermacto® (2.31%) was lighter (p<0.05) than control group (2.73%), while the liver weight of males fed Protexin® (1.88%), Fermacto® (1.98%) and Protexin®+ Fermacto® (1.97%) was lighter (p<0.05) than the control group (2.93%). In general, consumption of Fermacto® as an additive in basal diet of male Japanese quails caused decrease giblet and intestinal weight. Whereas, in quails fed Protexin®+ fermacto®, only in liver weights of both gender and weight of heart in males this reduction was existence. On the other hand, female

quails fed Protexin® caused increase in gizzard, intestines and heart weights. Food contamination, nutrient imbalance, environmental factors (stress) and mycotoxins are known to irritate gizzard and intestines of the gastrointestinal tract, thus causing an increase in the relative weights of these organs (Vahdatpour *et al.*, 2009). Liver weights were significantly heavier in birds that died from sudden death syndrome (SDS) and although this might be a reflection of the various diets of the field cases, it could be an indication of metabolic disease predisposing to SDS. Administration different levels of Protexin® in diet of broiler chickens decreased liver weight. The weight of internal organ weights except small intestine was significantly reduced by consumption of synbiotic ($p<0.01$) and prebiotic ($p<0.05$) (EL-Banna *et al.,* 2010). In agreement with this study, Bozkurt *et al.* (2009) reported that the liver and small intestine weights of male broilers with consumption of MOS prebiotic (Nutri-Mos®) decreased ($p<0.05$), but females were not influenced compare to control group. In another study, Azadegan Mehr *et al.* (2007) reported that Protexin® administration into the diet caused a significant decrease in liver relative weight of male broiler chicks ($P<0.05$). These results suggested that effects of both Fermacto® and Fermacto®+ Protexin® additives on the relative weight of internal organs were related to sex . Probably, due to existence of sexual hormones, males were sensitive to additives. Anti-microbial action for prebiotics and probiotics is an important reason for decreasing relative weight and pathological activity on internal organs in birds (EL-Banna *et al.,* 2010).

Table 3: Means visceral organs weight of quails (Percentage of live body weight) fed additives at 42 days of age

Diets (treatments)	Gizzard		Intestines		Heart		Liver	
	(percentage of live weight)							
	M	F	M	F	M	F	M	F
Basal diet (Control group)	1.91±0.27^{a}	1.76±0.20^{c}	2.82±0.75^{a}	3.30±0.70^{bc}	0.98±0.15^{a}	0.75±0.04^{b}	2.93±0.42^{a}	2.73±0.48^{a}
Basal diet +Protexin	1.98±0.22^{a}	1.96±0.41^{a}	2.88±0.99^{a}	4.83±0.28^{a}	0.89±0.04^{b}	0.90±0.04^{a}	1.88±.0.37^{b}	2.55±0.42^{ab}
Basal diet +Fermacto	1.83±0.14^{b}	1.86±0.13^{b}	2.43±0.23^{b}	3.01±0.85^{c}	0.81±0.00^{b}	0.71±.07^{b}	1.98±0.09^{b}	2.31±0.25^{b}
Basal diet + Protexin+fermacto	1.93±0.40^{a}	1.85±0.31^{b}	2.82±0.93^{a}	3.68±0.81^{b}	0.81±.07^{b}	0.83±0.14^{ab}	1.97±0.04^{b}	2.41±0.36^{b}

Means±standard deviation (n=8) within each column with no common superscript differ significantly ($p<0.05$).
M: mail; F: female

Hormones

The effects of feed additives intake on three growth-related hormones in Japanese quail are presented in Table 4. All male and female additive groups increased insulin level compared to control group. In female birds fed by additives and especially Fermacto®, insulin elevated as compared to control group ($p<0.05$). Furthermore, Consumption of Protexin®+ Fermacto® in males caused an elevation of insulin as compared to other groups ($p<0.05$). Consumption of each Protexin® and Fermacto® amount could elevate insulin in males that were not significant ($p>0.05$). The level of blood thyroxin (T_4) in males was not influenced by feed additives, but it was significantly ($p<0.05$) depressed in females by all feed additives as compared to control group. All male and female additive groups increased growth hormone level compared to control group. Clearly a significant ($p<0.05$) increase of growth hormone by addition of feed additive was observed in females but not in males. One can emphasize on the role of insulin in growth in respect to its role in entrance of five necessary amino acid to cells (phenylalanine, leucine, isoleucine, valine and tyrosine), and so, elevation of insulin in blood is positive for bird growth. Numerically, reduction of thyroxin could have been due to the positive effects of additives in alleviation of the negative effects of environmental condition (petty high temperature at summer) (Sabriea *et al.*, 2006). Li *et al.* (2007) showed that chito-oligosaccharide supplementation as prebiotic in broiler chicken diet resulted in significant ($P= 0.02$) elevation of growth hormone level as compared with the control group and chlortetracycline fed group at 42 days. In the study of Li *et al.* (2007), improvement values of BW, FI and FC were in harmony with growth hormone level, higher growth hormone and better growth performance were in prebiotic fed group. The results of present study about the influence of feed additives in increasing insulin and growth hormone levels and decreasing thyroxin hormone level are in agreement with the results of Teshfam *et al. (*2011).

Table 4: Means serum hormones of quails fed additives at 42 days of age.

Diets (treatments)	Insulin (µIU/ml)		Thyroxin (µg/dl)		Growth hormone (µIU/ml)	
	M	F	M	F	M	F
Basal diet (Control group)	8.15±0.87^{b}	6.58±0.42^{c}	2.78±0.59	2.35±0.60^{a}	0.40±0.11	0.45±0.17^{b}
Basal diet + Protexin	8.85±1.01^{b}	8.53±1.30^{b}	2.83±0.63	1.78±0.49^{b}	0.55±0.17	0.65±0.13^{a}
Basal diet + Fermacto	8.63±1.40^{b}	12.28±3.07^{a}	2.30±0.47	1.88±0.55^{b}	0.45±0.13	0.70±0.22^{a}
Basal diet + Protexin+ Fermacto	11.40±2.30^{a}	9.30±2.9^{b}	2.60±0.83	1.70±0.58^{b}	0.40±0.19	0.60±0.28^{a}

Means±standard deviation (n=8) within each column with no common superscript differ significantly ($p<0.05$).
M: mail; F: female

CONCLUSION

Based on the results, the supplementation of diets containing Fermacto® as a prebiotic and combination of Protexin®+ Fermacto® as a synbiotic improved quail's growth performance and elevated growth related hormones to producing healthy animal food products for human consumption. Thereby, stimulation of growth related hormones release can be a new theory for promotion of growth performance in poultry emphasis on quails. Definitively, more investigations are needed to confirm this theory.

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