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Effects of Feed Additives on Growth-Related Hormones and Performance of Japanese Quail (*Coturnix japonica*)

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Abstract: The objective of the present study was to determine the effects of probiotic, prebiotic and synbiotic as natural feed additives on growth performance, visceral organ weights in sulin, thyroxin and growth hormone (growth-related hormones) of Japanese quail. A total of 192, one day old Japanese quail chicks were randomly allocated to 1 of 4 treatments by 4 replicates and 12 birds pen^{-1}. The experimental diets consisted of a basal diet without additive (control), 0.2 g kg^{-1} probiotic (Protexin), 1.6 g kg^{-1} prebiotic (Fermacto) and 1.0 g kg^{-1} synbiotic (Biomin IMBO) added to the basal diet. At 42 days, growth performance values calculated then 8 birds (4 male and 4 female) randomly selected, weighted and slaughtered for visceral organs weight recording and blood sampling were taken for analysis of serum hormones by ELISA kits. Quails fed synbiotic and prebiotic had higher feed intake than control birds ($p<0.05$). High body weight and better feed conversion were in synbiotic ($p<0.05$) and then in prebiotic group. The weight of each visceral organs were lower in group that had prebiotic ($p<0.05$). The blood insulin in quails which had synbiotic was higher than other groups ($p<0.05$) and in probiotic and prebiotic groups were higher than control group ($p<0.05$). The growth hormone level was higher in synbiotic and probiotic fed groups than control group ($p<0.05$). In conclusion, these types of feed additives specially synbiotic and then prebiotic could be used in quail's diet to improve the body weight and feed conversion by increasing the feed intake and stimulating the insulin and growth hormone release and they have implications in human health in organic production of meat and egg.

Key words: Hormone, prebiotic, probiotic, quail, synbiotic, feed additives

INTRODUCTION

Recently, Japanese quail had gained attention in poultry industry as they are resistant to pathogens and a good producer of organic egg and meat for human healthy nutrition and they are being used as a beneficial animal model in researches (Minvielle, 2004; Huss *et al*., 2008; Curry, 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 development of drug-resistant bacteria (Sorum and Sunde, 2001), accumulation of drug residues in the carcas of the birds (Burgut, 1991), imbalance of normal microflora (Andremont, 2000) and finally harmful effects on human health (Abaza *et al*., 2008). 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. Recently, 3 types of novel feed additives/functional foods including probiotics, prebiotics and synbiotic are applied (Roberfroid, 2000). A probiotic was defined as a live microbial feed additive that beneficially affects the host animal by improving its intestinal micro floral balance (Fuller, 1989). 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 in the colon (Gibson and Roberfroid, 1995). The efficacy of probiotics may be potentiated by several methods. The selection of more efficient strains, gene manipulation, the combination of several strains and the combination of probiotics and synergistically acting components. This approach seems to be the best efficacy of use in these products is combination of both probiotics and prebiotics as synbiotic that may be defined as a mixture of probiotics and prebiotics which synergistically

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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 (Awad *et al*., 2009). 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, etc. (Chichlowski *et al*., 2007). Although, there are several hypotheses on the mode of action of feed additives on bird's growth and performance but this hypothesis are almost yet vague (Chen *et al*., 2009). Therefore, the present study was conducted to investigate the effects of a probiotic, a prebiotic and a synbiotic from novel types on Japanese quail's growth performance and three growth related hormones.

MATERIALS AND METHODS

Experimental design and housing: About 192 newly-hatched and healthy Japanese quail chicks from the Damavand quail co. flock mean body weight 7.78±0.39 g were provided in the present study in a specially designed farm in the city of Tabriz, Iran.

On the 1st day after hatching, chicks randomized placed in 4 treatment groups with 4 replicates and 12 chicks (48 birds group^{-1}) per all wiry cages (0.3×0.5×0.35 m) that were established with distance another at one room with controlled standard temperature, humidity, ventilation and 24 h of light (2.5 watt m^{-2}) kept until 42 days of age (Fig. 1).

Dietary treatments and additives: The basal diet (conventional mash) was formulated to meet and exceed the nutrient requirements of grower Japanese quails (NRC, 1994). Each feed additives in standard high level of manufactory recommendation was added to the basal diet and completely mixed and with water were available *ad libltum* from 1-42 days of age (Table 1). The 4 treatment groups were as follow:

Fig. 1: Experimental room of Japanese quail, Tabriz-Iran

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
Calculated analysis	
ME (Kcal kg^{-1})	2900.00
CP (%)	24.00
Calcium (%)	1.20
Available phosphorus (%)	0.55
Methionine (%)	0.57
Lysine (%)	1.47

*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

- Basal diet without additive (control group)
- Basal diet +200 g ton^{-1} protexin (as a probiotic, 2×10^{9} cfu g^{-1})
- Basal diet+1600 g ton^{-1} fermacto (as a prebiotic)
- Basal diet+1000 g ton^{-1} biomin IMBO (as a synbiotic, 5×10^{11} cfu kg^{-1})

Protexin is one of the novel probiotic used in poultry feed that is a multi-strain probiotic containing live microorganisms to establish, enhanced or re-establish essential micro flora in the gut.

Protexin is a highly concentrated pre mix containing seven strains of bacteria and two yeasts including: *Lactobacillus plantarum* 1.89×10^{10} cfu kg^{-1}, *Lactobacillus delbrueckii* ssp., *Bulgaricus* 3.09×10^{10}

cfu kg^{-1}, *Lactobacillus acidophilus* 3.09×10^{10} cfu kg^{-1}, *Lactobacillus rhamnosus* 3.09×10^{10} cfu kg^{-1}, *Bifidobacterium bifidum* 3.00×10^{10} cfu kg^{-1}, *Streptococcus salivarius* ssp., *Thermophilus* 6.15×10^{10} cfu kg^{-1}, *Enterococcus faecium* 9.85×10^{10} cfu kg^{-1}, *Aspergillus oryzae* 7.98×10^{10} cfu kg^{-}, *Candida pintoloppesii* 7.98×10^{10} cfu kg^{-1}. All the micro organisms in the protexin are naturally occurring and have been isolated from a wide rang of feed, plant, animal, bird and human sources (protexin; Nasrollah, 2009). Protexin probiotics in list of Generally Regarded as Safe (GRAS) of FDA are recorded (FDA). Fermacto is comprised of Aspergillus meal. Aspergillus meal is derived from an active fermentation of a primary *Aspergillus* sp. It is the mycelium contained in this totally killed fungi that allows the monogastric an expansion of its digestive capacity. The mycelium of the *Aspergillus* sp. is to support the healthy bacteria and allows it to multiply, producing high levels of short chain organic acids which may actually reduce the number of pathogenic bacteria. The mycelia cell wall of the *Aspergillus* sp. is composed of a high concentration of beta-glucans which demonstrate a very strong prebiotic stimulatory effect (fermacto). Biomin IMBO is a novel combination of the probiotic strain *Enterococcus faecium* (DSM 3530), prebiotic of fructo-oligosaccharides (derived from chicory) and immune modulating substances that derived from sea algae (Biomin IMBO).

Blood sampling and measurements: Feed Intake (FI) of each experimental unit (each cage) was recorded. At the end of experimental period (42 days) the total Body Weight (BW) of birds in each cage was measured and then Feed Conversation (FC) was calculation. At 42 days of age in fasting state (feed was removed 12 h before sampling) 1 male and 1 female bird from each cage (8 birds/treatment) were randomly selected, weighted, slaughtered and blood samples by cervical cutting and without anti coagulant for serum separation were collection. The total viscera, proventriculus, gizzard in testine, liver and heart were excised, weighted 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 system (AWAKNESS technology Inc., USA) including ELISA microplate reader stat fax 2100 and automatic ELISA plate washer (state fax 2006) for growth related hormones including: insulin (Biosource Inc, EASIA Kit. Belgium), growth hormone (Monobind Inc, ELISA Kit, USA) and thyroxin (T_4 ELISA Kit, Pishtaz Teb, Iran).

Statistical analysis: Analysis of Variance (ANOVA) using the 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 treatments at $p<0.05$ level (SAS, 2001).

RESULTS AND DISCUSSION

Growth performance: The effects of 3 different types of feed additives that were added to control basal diet on growth performance values are shown in Table 2. The Feed Intake (FI) of quails from 1-42 days of age was affected by experimental treatments ($p<0.05$). The addition of prebiotic and synbiotic significantly caused to FI elevation ($p<0.05$). Whereas, addition of probiotic to basal diet had no significantly effect on this parameter. The Body Weight (BW) at 42 days of age was affected by experimental treatments ($p<0.05$). The addition of synbiotic significantly increased BW ($p<0.05$). Although, the addition of prebiotic to basal diet numerically had show elevation. The Feed Conversion (FC) was significantly influenced by feed additives intake ($p<0.05$). Addition of synbiotic resulted in improvement ($p<0.05$) FC whereas, the prebiotic and probiotic had no influence on this parameter. By good management in all period groups faced no disease and mortality. Abaza *et al.* (2008) reported that it was clear that using bacillus subtilis and licheniform as probiotic in the experimental diet increased BW as compared to the control group by about 2.10% at 12 weeks ($p<0.05$). Whereas in FI had no significantly difference between groups at the end of experimental period (12 weeks) and FC was significantly improved in group having probiotic compared with control group at the same a ge ($p<0.05$). Hooge *et al.* (2004) found that bacillus subtilis spores as a probiotic direct feed significantly increased the broiler BW ($p<0.05$). 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

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

Diets (treatments)	Feed intake ($g\ bird^{-1}$)	Body wight (g)	Feed conversion ($g\ g^{-1}$)	Mortality (%)
Basal diet (control group)	697 ± 7^{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

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

Table 3: Means visceral organs weight of quails fed additives at 42 days of age

	Live weight (%)					
Diets (treatments)	Total viscera	Proventriculus	Gizzard	Intestines	Heart	Liver
Basal diet	10.43 ± 1.53^{a}	0.38 ± 0.07	1.83 ± 0.23^{b}	3.06 ± 0.72^{ab}	0.87 ± 0.09^{a}	2.83 ± 0.46^{a}
Basal diet + probiotic	10.63 ± 1.08^{a}	0.40 ± 0.04	1.97 ± 0.29^{a}	3.86 ± 0.64^{a}	0.89 ± 0.04^{a}	2.22 ± 0.40^{b}
Basal diet + prebiotic	9.34 ± 1.47^{b}	0.35 ± 0.03	1.85 ± 0.14^{b}	2.72 ± 0.76^{b}	0.76 ± 0.03^{b}	2.14 ± 0.17^{b}
Basal diet + synbiotic	11.23 ± 1.07^{a}	0.39 ± 0.04	2.00 ± 0.19^{a}	3.19 ± 0.61^{ab}	0.82 ± 0.05^{ab}	2.26 ± 0.28^{b}

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

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. Li *et al*. (2007) found that supplementation of chito-oligosaccharide (50 or 100 mg kg^{-1}) in broiler chicken diet during the starter phase (1-21 days) and overall experiment (1-42 days) improved BW, FI and FC ($p<0.05$) in comparison to birds fed the control diet. Awad *et al*. (2009) found that broiler chickens supplemented with the Biomin IMBO had a greater ($p<0.05$). BW compared with control birds whereas BW in the group fed probiotic (*Lactobacillus* sp.) were not significantly different. FC was lower for birds supplemented with synbiotic compared with control birds and bird's diets supplemented with probiotic. Although, probiotic supplemented birds had a lower FC than control birds. Cakir *et al*. (2008) concluded that addition of Biomin IMBO and other feed additives to basal diet of Japanese quail had no significant effect on each three growth performance values. Ahmad (2004) reported that poultry growth is promoted with the increasing doses of probiotic (Protexin) from 0.5-1.5 g 10 kg^{-1} feed. They could not detect any difference in FC of broilers as compared to control group.

Guclu (2003) reported that addition of Mannan Oligosaccharide (MOS) as prebiotic to quail diets at level of 0.75 and 1 g kg^{-1} reduces FI significantly ($p<0.01$) and enhances FC. Waldroup *et al*. (2003) stated that MOS improved FC significantly in turkey ($p<0.05$). 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) resulted 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 ($p<0.05$). In the present study, the effect of biomin IMBO as synbiotic on growth performance was better than other additives that could be a case of synergistic effects of probiotics and prebiotics components of this product. Then Fermacto as prebiotic containing aspergillus meal is the next important additives with positive effects on growth performance. Whereas, addition of protexin as probiotic to basal diet had no significant effect on these growth performance values in quails.

Visceral organ weight: The effects of feed additives intake on visceral organs weight in Japanese quail are shown in Table 3. The weights of total viscera, proventriculus, gizzard in testines, heart and liver that presented as percentage of live body weight, was significantly influenced by feed additives ($p<0.05$). The addition of prebiotic (Fermacto) to basal diet significantly reduce the total visceral weight in birds ($p<0.05$). Probably, this reduce is due to the presence of non-digestible fibers and its effects on gastrointestinal activity. Whereas, the weight of total viscera in birds fed probiotic and synbiotic additives were numerically higher than control group. This could be caused by the presence of live microbial organisms in two additives. Consumption of probiotic and synbiotic caused a significant increase in gizzard weight ($p<0.05$) compared with control group. Whereas, the prebiotical feed intake had no significant effect on gizzard weight. Hyper activity of gizzard muscle could have been one reason for this higher weight. Like total viscera weight, the intestine weight of group with prebiotic was lower. Although, difference between experimental groups and the control group was not significant but increase in the intestine weight by probiotic and decreasing of it by prebiotic was the reason for their significant difference between these groups ($p<0.05$). Consumption of prebiotic in basal diet caused a significant decrease in heart weight ($p<0.05$). These lower hearts weights in prebiotic group could be due to the lower heart load and/or promotion in ion balance for heart function and/or decrease in environmental vascular resistance and/or other reasons. The liver weight in response to each three additives was significantly reduced ($p<0.05$). One the hypothesis is lowering metabolic pressure on liver by using additives in feed, reduction of bile and protein synthesis in liver is another hypothesis. Awad *et al*. (2009) concluded that with adding biomin IMBO as synbiotic to basal diet, the weight of proventriculus, liver and cecum significantly decreased ($p<0.05$) and the weight of gizzard, small intestine and heart numerically decreased in comparison with the control group. Whereas they could not observe any significant effect on visceral organs weight with addition of *Lactobacillus* sp. as probiotic in basal diet of broiler chickens.

Although, Cakir *et al*. (2008) observed that addition of Biomin IMBO at a level of 1 g kg^{-1} of feed (equal to present study) had no significant effect on visceral organ weight in Japanese quails. Bozkurt *et al*. (2009) resulted that the weight of broilers liver with supplementation of probiotic (Primalac) and prebiotic (MOS) significantly was increased ($p<0.05$) and with supplementation of synbiotic (Primalac+MOS) numerically decreased whereas the other visceral organ weight had not been affected by these feed additives.

Eleftherios *et al*. (2010) reported that supplementation of diet with MOS as prebiotic causes significant ($p<0.05$) decrease in liver weight of Japanese quails.

In addition with attention to decreasing of total viscera and each visceral organ weight in quail fed by prebiotical diet, it can be concluded that probiotics can improve the carcass efficiency in quails and fermacto can be used for increasing the meat production efficiency.

Hormones: The effects of feed additives intake on 3 growth-related hormones in Japanese quail are shown in Table 4. The addition of prebiotic to basal diet of quails significantly elevated the insulin hormone level in serum ($p<0.05$) compared to control group (10.45 and 7.36 μIU mL^{-1}, respectively) and other groups. Furthermore, consumption of two other additives in diet caused significant increase in the levels of insulin compared to control group ($p<0.05$). One can emphasis on the role of insulin in growth in respect with 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.

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

Diets (treatments)	Insulin (μIU mL^{-1})	Thyroxin (μg dL^{-1})	Growth hormone (μIU mL^{-1})
Basal diet (control group)	7.36 ± 0.6^{c}	2.56 ± 0.3	0.43 ± 0.14^{b}
Basal diet + probiotic	8.69 ± 1.1^{b}	2.30 ± 0.5	0.60 ± 0.14^{a}
Basal diet + prebiotic	10.45 ± 2.2^{a}	2.09 ± 0.5	0.58 ± 0.16^{ab}
Basal diet + synbiotic	8.48 ± 1.5^{b}	2.08 ± 0.6	0.61 ± 0.19^{a}

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

Despite the fact that each three additives numerically decreased the thyroxin level but consumption of each feed additives by quails did not significantly changed the thyroxin hormone levels (T_4).

This result could have been due to the positive effects of additives, especially synbiotic in alleviation of the negative effects of environmental condition (petty high temperature at summer) (El-Soud *et al*., 2006).

The growth hormone significantly increased by consumption of feed additives ($p<0.05$), specially with use of synbiotic and probiotic in basal diet. Li *et al*. (2007) showed that by chito-oligosaccharide supplementation as prebiotic in broiler chicken diet resulted in the elevation of growth hormone level significantly ($p = 0.02$) compared with the control group and chlortetracycline fed group at 42 days of age.

Although, the IGF-I was constant in all groups. In study of Li *et al*. (2007), BW, FI and FC were in harmony with growth hormone level, higher growth hormone and better growth performance were in prebiotic fed group. Zhengkang *et al*. (2006) showed that by using isoflavonic phytoestrogens as new prebiotic, the growth hormones significantly increase ($p<0.05$) in sow colostrum but insulin was constant.

CONCLUSION

Based on the results, the positive effects of diets containing natural feed additives, especially synbiotics and prebiotics improved quail's growth performance and growth related hormones compared to the control diet. On the other hand with using these additives as growth promoters a healthier and organic meat and egg could be produced for human nutrition. In future, we have to try to concentrate to analyze the microbial populations in the GI tract and composition of the meat and egg in quails fed by additives.

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