J. BIOL. ENVIRON. SCI.,
2016, 10(28), 5-10

Original Research Article

Effects of Functional Feed Additives on Total Antioxidant Status, Lipid Peroxidation and Blood Indicators of Japanese Quails (*Coturnix coturnix japonica*)

Tohid Vahdatpour[1, 4*], Alireza Monadi-Sefidan[2], Naser Maheri-Sis[1] and Siamak Sandoghchian-Shotorbani[3]

[1]Department of Animal Sciences, Faculty of Animal and Veterinary Sciences, Shabestar Branch, Islamic Azad University, Shabestar, IRAN
[2]Department of Microbiology, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, IRAN
[3]Department of Immunology, Tabriz Branch, Islamic Azad University, Tabriz, IRAN
[4]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IRAN

Received: 04.12.2015; Accepted: 08.04.2016; Published Online: 21.07.2016

ABSTRACT

The purpose of present research was to investigate effects of a probiotic, a prebiotic and two synbiotics consumption on total antioxidant (TAO) capacity, malondialdehyde (MDA) level, percentages of heterophils, lymphocytes, heterophils /lymphocytes (H/L) ratio as a stress indicator, and albumin, globulin and albumin/globulin (A/G) ratio as a oncotic pressure indicator of blood in Japanese quails. The experimental diets consisted of a basal diet without additive (control), protexin® as a probiotic, fermacto® as a prebiotic, mixed of protexin® and fermacto® as a synbiotic, and Biomin-IMBO® as a other synbiotic, were added to the basal diet and with water were available *ad libitum* from 1 to 42 days of age of quails. The consumption of these additives had not significant effect on TAO capacity in females and on MDA in both genders, but consumption of prebiotic caused enhancement of TAO capacity of males ($P<0.05$). The H/L ratio of females fed by additives especially Biomin-IMBO® were lower comparison to control group ($P<0.05$). The blood albumin was enhanced in males fed by additives especially mixed of protexin® and fermacto® as synbiotic ($P<0.05$). The A/G ratio was increased by consumption of additives especially with each two synbiotics ($P<0.05$). The results show that the combination of probiotic and prebiotic as synbiotic is benefit additive for quails feeding. The synergetic effects of synbiotics in improving of live benefit microbial, absorption of necessary nutrients and providing of suitable environment in the gastrointestinal tract are important for health of blood indicators in Japanese quails.

Keywords: Antioxidant, Blood, Probiotic, Prebiotic, Synbiotic, Quail

INTRODUCTION

The free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the function of biomembranes, which can lead to the development of many pathological processes (Faix *et al.* 2003). Oxygen radicals and other reactive oxygen species (ROS) that widely produced by cell metabolism can react with double bonds of polyunsaturated fatty acids to yield lipid hydro-peroxides (Bulbul *et al.* 2008). Protective systems existing in the cells and blood avoid the excessive increment of undesirable oxidizers.

The oxidant-antioxidant status can vary between mammalian and birds because the birds have higher levels of blood uric acid than mammalian (Vahdatpour *et al.* 2011b). It is clear that uric acid lead to increasing of the total antioxidant (TAO) activity in birds. One of the major secondary oxidation products of per-oxidized polyunsaturated fatty acids is the malondialdehyde (MDA) that has been widely applied as the most common approach for the assessment of free radical damage and lipid per-oxidation in biological science (Bulbul *et al.* 2008). Japanese quail had gained attention in poultry industry, as good producer of organic egg and meat for human healthy nutrition and they are being used as a beneficial animal model in researches (Huss *et al.* 2008, Bishop 2009). 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. Three types of safe alternative functional additives including probiotics, prebiotics and synbiotic are applied (Roberfroid 2000, Chen *et al.* 2009). A probiotic was defined as a live microbial feed additive that beneficially affects the host animal by improving its intestinal micro-floral balance (Vahdatpour *et al.* 2011a). 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 (Roberfroid 2000). 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

* Corresponding author: vahdatpour@iaushab.ac.ir

defined as a mixture of probiotics and prebiotics 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 (Awad *et al.* 2009, 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. Little information is available regarding the effects of functional additives on physiological stress responses based on H/L ratio, colloid osmotic pressure of blood based on A/G ratio, TAO and MDA for oxidant-antioxidant status of Japanese quails. Therefore, the present study was conducted to investigate the effects of a probiotic, a prebiotic and two synbiotics on TAO capacity, MDA, percentage of Heterophils and Lymphocytes, H/L ratio, Albumin, Globulin levels and A/G ratio of blood in male and female Japanese quails.

MATERIALS AND METHODS

Experimental design and Housing

The 240 newly-hatched and healthy Japanese quail chicks from the Damavand Quail Company (body weight average, 7.69±0.23 gram) were provided and allocated in a specially designed room in the Tabriz, Iran. The experimental design was Completely Randomized Design (CRD). On the first day after hatching, chicks randomly placed in 5 treatment groups with 4 replicates and 12 chicks (48 birds/treatment) per wiry cages (0.2 m^2) that were established with distance another in controlled standard temperature, humidity, ventilation for quails housing and 24 h of light (2.5 watt/m^2) kept until 42 days of age.

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 high level of manufactory recommendation was added to the basal diet and were available *ad libitum* from 1 to 42 days of age. The four treatment groups were as follow:

Basal diet without additive (control group)
Basal diet + 200 mg/kg Protexin® (as a probiotic)
Basal diet + 1600 mg/kg Fermacto® (as a prebiotic)
Basal diet + 100 mg/kg Protexin® + 800 g/ton Fermacto® (as a synbiotic 1)
Basal diet + 1000 mg/kg Biomin-IMBO® (as a synbiotic 2)

Protexin® is one of the novel probiotic used in poultry feed that is a multi-strain probiotic containing live microorganisms to establish, enhanced or reestablish 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, *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* 9.85×10^{10} cfu/kg, *Aspergillus oryzae* 7.98×10^{10} cfu/kg, *Candida pintoloppesii* 7.98×10^{10} cfu/kg. All the microorganisms in the protexin® are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources. Protexin® probiotics in list of Generally Regarded as Safe (GRAS) are recorded (FDA). Fermacto® is comprised of *Aspergillus meal* that 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

At 42 days of age in fasting state (feed was removed 12 h before sampling) one male and one female bird from each cage (8 birds/treatment) were randomly selected and blood samples were taken from wing vein. The separated sera were analyzed using ParsAzmun kits for TAO, albumin and globulin in auto analyzer (ALCYON 300) by international federation of clinical chemistry methods (IFCC 2011). One droplet blood was obtained via wing vein. Blood smears prepared and air dried the stained in concentrated May-Grunwald stain for 6 min, 1:1 May-Grunwald stained stilled water for 1.5 min and 1:9 Geimsa stain for 15 min (Robertson and Maxwell 1990). In order to counting of heterophils and lymphocytes, 100 cells per film were examined by light microscopy. The results are presented as the percentage of each cell occurring in each film. Finally, the H/L and A/G ratio were calculated. For MDA assay, one ml of each supernatant was added to a mixture containing three ml of phosphorous acid (1%) and 1 ml of thiobarbituric acid (TBA, 0.67%) in an aqueous solution.

The reaction mixture was heated for 60 min up to 90°C, and then was cooled in a room temperature. Then, 3 ml of N-butanol was added to each tube, and the tubes were shaken and then centrifuged. The absorbance of N-butanol phase was measured spectrophotometrically at 532 nm and the amount of thiobarbituric acid reactant substances (TBARS) was calculated from a calibration curve and reported as nmol MDA/mg.

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)	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.

Statistical Analysis

Statistical Analysis performed using of SAS software base on Completely Randomized Design (CRD) with the GLM procedure, Bartlett's test for homogeneity of data variance and standard deviation were calculated. Duncan's new multiple range tests was used to compare the means treatments and P value of lesser than 0.05 was considered statistically significant (SAS Institute 1994).

RESULTS AND DISSCUSSION

Total antioxidant (TAO) and Malondialdehyde (MDA)

The effects of four different types of feed additives on TAO and MDA are presented in table 2. Consumption of prebiotic caused to enhancement of TAO in males (P<0.05); where probiotic and compounded additives (synbiotic 1 and 2) had no effect on TAO. Consumption of these additives not has been significant changes on MDA in both genders and also TAO in females.

Probiotic research was first originally from Metchnikoff's research on the relationship between life prolongation and eating yogurt (Zhang 2010). Antioxidant effect of probiotics may be an important mechanism involved in its function such as inflammatory response because both local and systemic inflammatory responses are associated with the production of ROS (Zhang *et al.* 2010). Mikelsaar and Zilmer (2009) showed that *lactobacillus fermentum* ME-3 has been not only demonstrated to possess high total antioxidant activity of intact cells *in vitro*, but also it can increase the anti-oxidative activity of sera and improved the composition of low-density lipoproteins (LDL) *in vivo*. Functional food played the bioavailability of antioxidants, which is considered as an important key-factor in the biological activity of substances in the gastrointestinal tract and their absorption through the intestinal walls into the blood circulation system (Nikpiran *et al.* 2013). Prebiotics have the ability to scavenge the free radicals of the body as well as minimize the lipid peroxidation and overcome the mutagenic and carcinogenic effects of MDA. Therefore the determination of lipid peroxidation is a first step toward assessing the oxidative damage in most tissue. Functional foods are beneficial dietary supplement for improving antioxidant activities, decreasing lipid peroxidation levels which are important in evaluating the efficiency of commercial poultry production system that is influenced primarily by the quality of feed and feed intake (Heba *et al.* 2014). In present study, prebiotic consumption caused to TAO elevation and MDA depression. This may be due to the antioxidant effect of beta-glucans structure on bacteria and producing of high levels of short chain fatty acids. These suggested that prebiotic consumption comparison to the other additives could be better protecting quails from oxidative stress.

Table 2. Effects of functional additives on total antioxidant (TAO) and malondialdehyde (MDA) of quails.

	Male						Female					
Parameters	Con	Pro	Pre	Syn1	Syn2	P-value	Con	Pro	Pre	Syn1	Syn2	P-value
TAO (mmol/l)	0.73^{b}	0.89^{ab}	1.18^{a}	0.86^{ab}	0.79^{b}	0.05	0.65	0.70	0.60	0.64	0.75	0.80
MDA (µmol/l)	23.98	22.24	18.10	18.38	28.06	0.81	23.75	28.61	26.74	33.00	33.21	0.22

Means (n=8) within each rows with different superscript have significantly difference at its rows P-value.
Con: Control, Pro: Probiotic of protexin, Pre: Prebiotic of fermacto, Syn1: Synbiotic of protexin+fermacto, Syn2: Synbiotic of Biomin-IMBO.

Heterophils, Lymphocytes and H/L ratio

The effects of feed additives intake on heterophils (H), lymphocytes (L) and H/L ratio of Japanese quails are presented in table 3. Consumption of these feed additives had not significant effect on heterophils, lymphocytes and H/L ratio in males. In females, Heterophils and Lymphocytes counts were not be affected by additives but it showed that H/L ratio of females was significantly declined by additives specially synbiotic 2 comparison to control group (P<0.05). In poultry H/L ratio is better and stable index for assess of stress level (Vahdatpour *et al.* 2009). Karoglu and Drudag (2005) found that adding of probiotic to broilers diet could be inhibited the nutritional stress or any stress which causes an increase in H/L ratio. Ghareeb *et al.* (2008) reported that Lymphocytes counts increased (P<0.05) for both probiotic and prebiotic treated group compared with the control. Moreover, the H/L ratio decreased significantly in additives fed groups compared to control group. Because the stress could cause an increase in the stimulation of adrenal gland to produce of stress hormones which have a direct effect to lymphatic nodes and causes an H/L ratio elevation. Therefore, these results indicate that help to overcome stress due to transportation with lesser physiological response.

Table 3. Effects of functional additives on Heterophils (H), Lymphocytes (L) and H/L ratio of quails.

	Male						Female					
Parameters	Con	Pro	Pre	Syn1	Syn2	P-value	Con	Pro	Pre	Syn1	Syn2	P-value
H (%)	19.00	23.00	24.00	19.00	18.00	0.25	24.00	19.00	17.33	17.00	14.67	0.29
L (%)	75.67	72.33	71.33	76.67	77.33	0.32	67.67	73.00	77.00	76.00	77.33	0.24
H/L	0.25	0.31	0.32	0.25	0.24	0.30	0.36^{a}	0.26^{b}	0.23^{b}	0.24^{b}	0.19^{c}	0.04

Means (n=8) within each rows with different superscript have significantly difference at its rows P-value.
Con: Control, Pro: Probiotic of protexin, Pre: Prebiotic of fermacto, Syn1: Synbiotic of protexin+fermacto, Syn2: Synbiotic of Biomin-IMBO.

Albumin, Globulin and A/G ratio

The effects of feed additives intake on Albumin (A), Globulin (G) and A/G ratio of Japanese quails are presented in table 4. In male quails which fed by additives especially synbiotic 1, Albumin level was improved. Globulin was reduced by using synbiotic 1 and 2. Therefore, A/G ratio as a oncotic indicator was elevated by adding of additives specially synbiotics. In females by adding synbiotic 1 and 2, Albumin level is elevated and consumption of synbiotic 1 cause to significantly elevation of A/G ratio and Globulin level wasn't affected by adding of these types of functional additives. Khosravi *et al.* (2008) reported that consumption of some additives such as protexin had not significant effects on total protein, albumin, globulin and A/G ratio in broilers.

Therefore, these functional foods especially synbiotics affect on A/G ratio as well as during periodic decrease in immune response and enhancement of oncotic pressure. Immunity plays a key role in maintaining animal health, and thereby achieves an adequate, required level of performance. Immunity can be stimulated by various methods and preparations. Although, in an indirect way can be determining the effectiveness of the immunological system of quails.

Table 4. Effects of functional additives on Albumin, Globulin and A/G ratio of quails.

	Male						Female					
Parameters	Con	Pro	Pre	Syn1	Syn2	P-value	Con	Pro	Pre	Syn1	Syn2	P-value
A (g dL^{-1})	1.58^{c}	1.70^{b}	1.90^{ab}	2.08^{a}	1.83^{ab}	0.00	2.10^{b}	1.93^{b}	2.25^{ab}	2.43^{a}	2.53^{a}	0.00
G (g dL^{-1})	1.62^{a}	1.20^{b}	1.20^{b}	0.80^{c}	0.83^{c}	0.00	1.72	1.93	1.48	1.32	1.74	0.06
A/G	0.98^{c}	1.42^{b}	1.59^{b}	2.66^{a}	2.23^{a}	0.00	1.23^{bc}	1.00^{c}	1.53^{ab}	1.83^{a}	1.46^{b}	0.00

Means (n=8) within each rows with different superscript have significantly difference at its rows P-value.
Con: Control, Pro: Probiotic of protexin, Pre: Prebiotic of fermacto, Syn1: Synbiotic of protexin+fermacto, Syn2: Synbiotic of Biomin-IMBO.

CONCLUSION

Based on results, the consumption of prebiotic of fermacto improved total antioxidant capacity of male quails. Heterophil/Lymphocyte ratio as a main and stable stress indicator was declined in additive group's intake specially by adding synbiotic of Biomin-IMBO to basal diet. Also consumption of synbiotic of protexin+fermacto enhanced albumin and each synbiotics increased Albumin/Globulin ratio. Therefore, consumption of additives specially synbiotics and prebiotic may aid in overcome any deficiency and enhance of blood indicators that show tolerance to stress concomitantly and resistance for metabolic disorders case to oncotic pressure indicator enhancement in quails flock.

ACKNOWLEDGMENTS

The presented results in this article were part of the research project that conducted in Drug Applied Research Center (Tabriz University of Medical Sciences): (www.darc.hbi.ir). The authors would like to thank Damavand Quail Co. (www.dquail.ir) Tehran, Iran for equipments of the Research Room of Japanese Quail in Tabriz.

REFERENCES

Attia Y, Zeweil HS, Alsaffar AA, El-Shafy AS (2011) Effect of non-antibiotic feed additives as an alternative to flavomycin on broiler chickens production. Archiv für Geflügelkunde 75: 40-48.

Awad WA, Ghareeb K, Abdel-raheem S, Bohm J (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. Poultry Science 88: 49-55.

Bishop BC (2009) Animal models used in identifying gender-related differences, International Journal of Toxicology 20: 153-160.

Bulbul A, Bulbul T, Kucukersan S, Sireli M, Eryavuz A (2008) Effects of dietary supplementation of organic and inorganic Zn, Cu and Mn on oxidant/antioxidant balance in laying hens. Kafkas Universitesi Veterinar Fakultesi Dergisi 14: 19-24.

Cakir S, Midilli M, Erol H, Simsek N, Cinar M, Altintas A, Alp H, Altintas L, Cengiz O, Antalyali A (2008) Use of combined probiotic-prebiotic, organic acid and avilamycin in diets of Japanese quails. Revuede Medecine Veterinaire 11: 565-569.

Chen KL, Kho WL, You SH, Yeh RH, Tang SW, Hsieh CW (2009) Effects of bacillus subtilis var natto and saccharomyces cerevisiae mixed fermented feed on the enhanced growth performance of broilers. Poultry Science 88: 309-315.

Faix S, Faixova Z, Michnova E, Varady J (2003) Effect of per os administration of mercuric chloride on peroxidation processes in Japanese quail. Acta Veterinaria Scandinavica 72: 23-27.

Heba AM, Abd El-Kadera A, Sally Alama S, Abeer Nafeaa A, Karima B, Mahrous F (2014) Influence of dietary supplementation of mannanoligosaccharide (Bio-Mos®) prebiotic on the genotoxic and antioxidant status in japanese quail. International Journal of Pharmaceutical Sciences Review and Research 27: 289-295.

Huss D, Poynter G, Lansford R (2008) Japanese quail (Coturnix japonica) as a laboratory animal model, Laboratory Animals 37: 513-519.

Karoglu M, Durdag H (2005) Effect dietaryprobiotic (Sacchromyces cerviciae) ofadding Galli acid Iraq probiotic on some physiological properties and intestinal micro flora in Broiler chicks. Proceeding of second Veterinary Scientific Conference 41: 102-115.

Khosravi A, Boldaji F, Daster B, Hasani S (2008) The use of some feed additives as growth promoter in broiler nutrition. International Journal of Poultry Sciences 7: 1095-1099.

Mikelsaar M, Zilmer M (2009) Lactobacillus fermentum ME-3 an antimicrobial and antioxidative probiotic. Microbial Ecology in Health and Disease 21: 1-27.

Nikpiran H, Vahdatpour T, Babazadeh D, Vahdatpour S (2013) Effects of saccharomyces cerevisiae, thepax and their combination on blood enzyme and performance of japanese quails (coturnix japonica). The Journal of Animal and Plant Science 23: 369-375.

NRC (1994) Nutrition requirements of poultries, National Academy Press, Washington DC.

Roberfroid MB (2000) Prebiotics and probiotics: are functional foods?. The American Journal of Clinical Nutrition 71: 1682-1687.

Robertson GW, Maxwell MH (1990) Modified staining techniques for avian blood cells. British Poultry Science 31: 881-886.

SAS institute (2001) SAS user‘s Guide: Statistic Version 8.2, SAS Institute lnc. CARY NC USA.

Vahdatpour T, Nazeradl K, Ebrahimnezhad Y, Mahery sis N, Riyazi SR Vahdatpour S (2009) Effects of corticosterone intake as stress-alternative hormone on broiler chickens: performance and blood parameters. Asian Journal of Animal and Veterinary Advances 4: 16-21.

Vahdatpour T, Nikpiran H, Ahmadzadeh A, Riyazi SR, Vahdatpour S (2011a) Effects of active, inactive and compounded saccharomyces cerevisiae on growth-related hormones and performance of Japanese quails (*Coturnix Japonica*), African Journal of Biotechnology 10: 15205-15211.

Vahdatpour T, Nikpiran H, Babazadeh D, Vahdatpour S, Jafargholipour, MA (2011b) Effects of Protexin®, Fermacto® and combination of them on blood enzymes and performance of Japanese quails (Coturnix Japonica). Annals of Biological Research 2: 283-291.

Zhang Y, Du R, Wang L, Zhang H (2010) The antioxidative effects of probiotic Lactobacillus casei zhang the hyperlipidemic rats. European Food Research and Technology 231: 151-158.