INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-4, Issue-2, April-June-2014
ISSN 2231-4490

Coden : IJPAES www.ijpaes.com
Received: 28th Feb-2014 *Revised: 29th March-2014* *Accepted: 2nd April-2014*
Research article

EFFECTS OF DIETARY FUNCTIONAL ADDITIVES ON CHARACTERISTICS AND MINERALS OF TIBIA BONE AND BLOOD PARAMETERS OF JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*)

Tohid Vahdatpour[1*], Yahya Ebrahimnezhad[1] and Sina Vahdatpour[2]

[1]Department of Animal Sciences, Faculty of Animal and Veterinary Sciences, Shabestar Branch, Islamic Azad University, Shabestar, Iran
[2]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
*Corresponding Author: Dr. Tohid Vahdatpour, Department of Animal Sciences, Islamic Azad University, Shabestar Branch, Shabestar - Iran
Email: vahdatpour@iaushab.ac.ir Tel: +984114777882 Fax: +984114777840

ABSTRACT: The aim of present study was to evaluate the comparison influences of probiotic, prebiotic and synbiotic on physical characteristics and minerals of tibia and related blood parameter of Japanese Quails. Total of 192 one-day old Japanese quail chicks were randomly assigned in four treatments and four replicates. The experimental diets consisted of a basal diet without additive (Control), probiotic (Protexin®), prebiotic (Fermacto®) and synbiotic (Biomin®IMBO) added to the basal diet. At the end of Experiment, blood samples were taken and then birds slaughtered for tibia bone analysis. The synbiotic consumption, caused to elevation of alkaline phosphates activity ($p<0.05$) in males. But in females, enzyme activity was depressed by consumption of additives ($p<0.05$). In males, the tibia length/tibia weight indicator, increased by additives consumption ($p<0.05$). Diaphysis diameter in relative to body weight with use of additives declined in males and elevated only in probiotic fed in females group ($p<0.05$). The medullar canal diameter was greater than other groups ($p<0.05$) in females fed by probiotic. The wall thickness of midpoint decreased in males of prebiotic fed group and in females of probiotic fed group ($p<0.05$). The calcium and phosphorous of tibia bone structure were elevation in males fed by synbiotic ($p<0.05$). The robusticity index in males had been show elevation by additive consumption ($p<0.05$). Results indicated that response of birds to additives is dependent to gender and synbiotic is a more affective to bone parameters. But, all of these effects do not positive for bone characteristics. Therefore, the more researches are necessary to clearing for reasons of some negative effects.

Key words: Additive, Tibia bone, Mineral, Quail

INTRODUCTION

One of needs and important goal of poultry industries is production of organic meat and eggs. Therefore functional additives uses including probiotics, prebiotics and synbiotics have gained widespread and replaced to antibiotics [9, 16]. Probiotics are viable, defined micro organisms in sufficient numbers, witch after the micro flora in a compartment of the host and by that exert beneficial health effects in the host. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of 1 or a limited number of bacteria (probiotics) in the gastrointestinal tract and there by exert a health-promoting effect. A synbiotic is a product containing prebiotics and probiotics and in which the prebiotic compound selectively favors the probiotic compound [6, 16]. Currently, there have been some confecting studies; functional additives have been shown to improve performance parameters in poultry [5]. Although modes of acting are not entirely clear especially in some body systems. Currently, there are two main research concerns relating to skeletal disorders in poultry that cause to economical and production losses including: osteoporosis in laying hens and leg disorders for example tibial dyschondroplasia in rapid bone growth broiler (meat – type) [3]. Leg and gait disorders have been a considerable problem for the poultry industry. Sever defects greatly impair the walking ability of birds, leading to mortality from starvation and dehydration, but even mild deformities have been show to cause discomfort, pain and lameness [8].

Nutritional approaches have improved the situation, there remains scope for further improvement. Many nutritional factors can an affect on bone characteristics and composition. The skeleton as dynamic tissue not only provides structural support for bird but is an important mineral source for metabolic needs. The fit safety of organic and inorganic substances and controlling of other nutritional factors (toxins and antinutrients) are important for bones health that affected by some unknown nutrient factors in functional additives [3, 6, 9]. By invasive methods recording of some parameters including: bone weight, bone length, bone ash and diaphysis diameter and exist to determine the bone characteristics, mineralization and indicators in poultry [9]. The aim of present study was to investigate the effects of functional additives that add to diet of Japanese quails on the tibial bone health including: physical characteristics, density indicators and alterations in related blood parameters.

MATERIALS AND METHODS

Experimental design and housing: present study was conducted at Islamic Azad University, Shabestar Branch, Iran in summer of 2012. A total of 192 one-day old Japanese quail chicks were provided from the Damavand quail Co. flock and randomly assigned in 4 treatments, 4 replicate with 12 birds per replicate (16 pen). The experimental design was Completely Randomized Design (CRD). 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) (Figure 1).

Treatments and additives: Nutrients compositions of diets for quails at 1 to 42 days old were based on the National Research Council [10] 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 Biomin®IMBO (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 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
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.

Blood sampling and measurements: At the end of Experiment (42d) in fasting state, blood samples were collected from wing vein of two birds (1 male and 1 female) per pen and rapidly were centrifuged at 5000 rpm during 5 minutes and then sera by using commercial Kits (pars azmun, Iran) were assayed for calcium, phosphorus, Magnesium and alkaline phosphates enzyme by international federation of clinical chemistry methods [4] and Auto analyzer system (ALCYON 300). Then, quails killed by cervical cutting. Each two femurs and tibiotarsal samples were removed and cleaned with flesh intact. The drumsticks were labeled by plastic designed numbers and immersed in boiling water for 15 minutes (figure 2). After cooling, the drumsticks were de-fleshed by hand and tibial bones with out muscles, ligament and tendons were obtained. Tibia bones were air- dried for 37 h at room temperature. The lengths and weight of the bones were measured using digital caliper and scale. Each bone was marked at midpoint and carefully cutting from mid-shaft of bone by a saw-knife. The outside and inside diameters (maximum and minimum) were measured perpendicular using digital caliper (Figure 3).

Figure 1: Experimental room of Japanese quail, Tabriz-Iran (Instituted by Dr. Vahdatpour)

Figure 2: Labeled of the cleaned tibia bones

Figure 3: Measuring of tibia dimensions using digital caliper

The thicknesses of the walls were calculated from to subtract of medullar canal diameter from diaphysis diameter. The bone weight/length index was obtained by dividing the tibia weight by its length) [14]. The tibiotarsal and the robusticity indexes are determined using the following formulas, respectively:

Tibiotarsal index = diaphysis diameter – medullary canal diameter/diaphysis diameter × 100 [1]

Robusticity index = bone length/ cube root of bone weight [12]

To determine bone ash content, bones were oven-dried at 105°C for 24 h and in an electrical furnace at 600°C for 6 h. The percentage of ash was determined relative to dry weight of the tibia. The ashes were dissolved in 2.5 ml hydrochloric acid (water solution 1:1) and diluted to 50 ml using redistilled water. The content of calcium, inorganic phosphorous and magnesium in bones ash were determined by commercial kits (pars azmun, Iran).

Statistical analysis: The data of experiment were analyzed by an analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS [13] and means were compared by Duncan`s multiple Range test at p<0.05 level [2].

RESULTS

The effects of additives on blood parameters and tibia ash of male and female quails are presented in Table 2. In this study, it has not seen any dietary treatment effect on blood calcium and phosphorous. The blood alkaline phosphates enzyme affected by dietary treatments (p<0.05). Consumption of synbiotic caused to enzyme activity elevation (p<0.05) in males. But in females enzyme activity was depressed by consumption of additives (p<0.05). The effects of feed additives on physical characteristics of male and female quails are presented in table 3 (as percentage of live body weight) and table 4 (as percentage of tibia weight). The length and weight of tibia bone as percentage of body weight were not affected by dietary treatments (p>0.05). In males, the tibia weight, increased by additives consumption, especially by synbiotic (p<0.05). Whereas, this parameter do not affected in females.

Table 2: The biochemical parameters of blood and tibia bone in quails fed additives at 42 d

	Male				Female			
Parameters	Control	Probiotic	Prebiotic	Synbiotic	Control	Probiotic	Prebiotic	Synbiotic
Blood Ca (mg/dl)	7.73±0.8	7.73±0.72	7.25±0.90	6.5±0.71	19.30±2.15	17.23±3.90	15.98±2.90	22.93±3.95
P (mg/dl)	8.53±0.91	7.78±0.76	7.58±0.88	7.30±0.71	12.03±1.20	11.45±1.11	12.93±1.18	13.34±1.29
Alp (IU/L)	1314±181^{b}	1513±201^{b}	1335±208^{b}	1816±220^{a}	2315±240^{a}	1713±217^{b}	1463±281^{b}	1601±279^{b}
Tibia ash Ca (mg/g)	155±8^{b}	162±11^{b}	156±9^{b}	170±13^{a}	165±11	160±14	166±9	170±10
P (mg/g)	64±4^{b}	66±4^{b}	68±6^{b}	78±8^{a}	723±7	68±8	78±9	79±9
Mg (mg/g)	6.8± 0.3	6.9±0.2	7.2±0.7	7.5±.09	7.4±0.7	7.2±0.5	7.9±0.5	7.8±0.8

Means±standard deviation within each row with no common superscript differ significantly (P<0.05).

Table 3: The physical characteristics of tibia bone of quails that presented as live body weight percentage of each bird sample at 42 d

	Male				Female			
Parameters	Control	Probiotic	Prebiotic	Synbiotic	Control	Probiotic	Prebiotic	Synbiotic
Length (%)	27.80±2.9	27.39±3.1	25.17±2.2	25.97±2.5	22.14±2.0	24.00±2.2	22.58±2.3	22.97±1.9
Weight (%)	0.63±0.05	0.61±0.03	0.56±0.04	0.54±0.04	0.51±0.04	0.53±0.02	0.53±0.03	0.56±0.06
D. D. (%)	1.57±0.12^{a}	1.47±0.11^{b}	1.45±0.15^{b}	1.40±017^{b}	1.27±0.12^{b}	1.41±0.18^{a}	1.29±0.15^{b}	1.31±0.17^{b}
M. C. D. (%)	0.95±0.03	0.93±0.04	0.98±0.05	0.86±0.04	0.68±0.04^{b}	1.70±0.06^{a}	0.75±0.05^{b}	0.73±0.04^{b}
M. W. T. (%)	0.62±0.07^{a}	0.53±0.04^{ab}	0.47±0.06^{b}	0.54±0.02^{ab}	0.58±0.05^{a}	0.34±0.04^{b}	0.54±0.06^{a}	0.58±0.04^{a}

DD. Diaphysis Diameter; M. C. D. Medullar canal Diaphysis; M. W. T. Midpoint Wall Thickness
Means±standard deviation within each row with no common superscript differ significantly (P<0.05).

The percentage of ash and diaphysis diameter that presented as percentage of tibia weight was not affected by dietary treatments. Diaphysis diameter in relative to body weight with use of additives declined in males and elevated only in probiotic fed in females group ($p<0.05$). Medullar canal diameter does not affected by additives consumption in males. But, in females fed probiotic medullar canal diameter was greater than other groups ($p<0.05$). The wall thickness of midpoint decreased in males of prebiotic fed group and in females of probiotic fed group ($p<0.05$). The calcium and phosphorous of tibia bone structure were elevation in males fed by synbiotics ($p<0.05$). The calcium and phosphorous in females and magnesium in each two gender were not affected by dietary treatments. The calculated physical indexes of tibia bone in male and female quails are presented in table 5.The robusticity index in males significantly had been show elevation by additive consumption especially synbiotic ($p<0.05$). The Tibiotarsal and Seedor Indexes not affected by dietary treatments.

Table 4: The physical characteristics of tibia bone of quails that presented as percentage of tibia weight at 42 d

	Male				Female			
Parameters	Control	Probiotic	Prebiotic	Synbiotic	Control	Probiotic	Prebiotic	Synbiotic
Length (%)	81.15±4.5^{c}	89.58±5.0^{b}	90.48±4.8^{b}	96.12±5.9^{a}	88.00±4.4	91.25±5.0	84.41±4.7	83.85±4.1
Ash (%)	44.8±1.6	45.64±1.1	45.06±3.2	47.09±2.5	52.95±1.8	50.33±1.1	52.81±4.1	54.01±2.8
D. D. (%)	5.65±0.91	5.34±0.87	5.67±0.77	5.39±0.68	5.71±0.80	5.87±0.87	5.75±0.91	5.67±0.87
M. C. D. (%)	4.42±0.51	4.34±0.58	4.72±0.31	4.34±0.33	4.40±0.40	5.16±0.51	4.52±0.48	4.26±0.54
M. W. T. (%)	2.22±0.18^{a}	1.96±0.22^{ab}	1.88±0.21^{b}	2.07±0.28^{ab}	2.65±0.19^{a}	1.43±0.13^{b}	2.42±0.19^{a}	2.50±0.24^{a}

DD. Diaphysis Diameter; M. C. D. Medullar canal Diaphysis; M. W. T. Midpoint Wall Thickness
Means±standard deviation within each row with no common superscript differ significantly ($P<0.05$).

Table 5: The calculated physical indexes of tibia bone of quails at 42 d

	Male				Female			
Indexes	Control	Probiotic	Prebiotic	Synbiotic	Control	Probiotic	Prebiotic	Synbiotic
Robusticity	2.10±0.12^{c}	2.61±0.15^{b}	2.72±0.14^{b}	3.15±0.11^{a}	2.49±0.08^{b}	2.85±0.14^{a}	2.20±0.15^{c}	1.98±0.10^{c}
Tibiotarsal	39.83±4.11	36.59±3.98	33.45±3.18	37.00±3.01	44.00±4.19	22.66±4.18	38.76±4.95	44.75±3.95
Seedor	1.26±0.11	1.12±0.15	1.11±0.21	1.04±0.22	1.15±0.18	1.10±0.18	1.19±0.16	1.23±0.20

Means±standard deviation within each row with no common superscript differ significantly ($P<0.05$).

DISCUSSION

Quails like to chickens are characterized by in a fast growth rate. There are two main researches relating to skeletal disorders in poultry: osteoporosis in egg-laying type and leg disorders caused by rapid bone growth in meat-type. There are numerous causes of leg-bone abnormality. Severe defects greatly impair the walking ability of birds, leading to mortality from starvation and dehydration [9]. But even mild deformities have been shown to cause discomfort or pain and finally decreasing of performance parameters. The mixtures used in nutrition are expected to have a high concentration of protein, energy, vitamins and minerals supplemented with stimulators [7]. Recently, functional additives as growth stimulators have replacing with traditional stimulators such as Antibiotics [6]. Probiotics, prebiotics and synbiotics on the base of structures have different effects on bone relevant parameters. It was show that prebiotics stimulated the absorption of bone-relevant minerals such as calcium, magnesium and zinc in short-term experiments. But, in present study, the Ca, P of blood and mg of bone not affected. In present study, the Ca and P of bone structure elevated by synbiotic consumption only in males. The Alkaline phosphates enzyme in synbiotic intake group was higher than other groups in males. Mutus et al (2006) reported that consumption of probiotic including bacillus lichniformis and bacillus subtilis not affected the mineral content of tibia bone [9]. Katharina et al (2013) reported that synbiotics stimulated the facial bacterial counts of lactobacilli and bifidobacteria in human subjects [6]. But little effect was seen when only the probiotic (bifidobacterium lactis HN019) or the prebiotic (galactooliyosascharides) was given. Such observation may allow one to assume a more effective stimulation of mineral absorption by synbiotic compared with prebiotics or probiotics alone. Mutus at al (2006) reported that birds fed the probiotic diet had greater medial and lateral wall thickness of the tibiotarsal [9]. Whereas, birds fed with the control diet had greater medullar canal diameter. In present study, probiotic fed groups show greater diaphysis diameter in males and in additives fed group lesser in females.

Medullar canal diameter in probiotic fed group was greater than other groups in males. Also most research reported that probiotic diet resulted in a greater tibiotarsal index. Inversely, in present study this parameter was no significant in dietary treatments [15]. Mutus et al (2006) reported that tibiotarsal weight and length, tibiotarsal weight/ length index, robusticity index, diaphysis diameter were not affected by the probiotic diet [9]. In present study, the length of tibia (percentage of tibia weight) was long in male quails fed additives especially in synbiotic group. In present study the robusticity index and diaphysis diameter affected by dietary treatments. The low robusticity index indicates a strong bone structure [12]. On the contrary to most study, in present study the three indexes showed that mineralization and strong of bone depressed by feeding of additive. Especially Robusticity index with consumption of additives increased in males. In females, probiotic consumption caused to elevation and prebiotic and synbiotic consumption caused to depression. With attention to elevation of tibial ash, Ca and P in synbiotic group in present study and the report of Mutus et al (2006) my be resulted that synbiotics are benefit for improvement of the bones mineralization. According to these result, it is not been a well established link between probiotics and prebiotics with mineral absorption or bone development. In conclusions the effects of synbiotic preparation is affected by the same aspects mentioned in bone characteristics and mineral absorption for probiotics and prebiotics. These effects were not in all cases uniform. Longitudinal growth of bone especially in males is important effect of additives. But consumption of additives in quails has not shown positive effects on bone indexes and strength. However, little is known in this field and future research is required.

ACKNOWLEDGMENT

This article summarized from the research project (number: 51955900401005) conducted in "Shabestar branch, Islamic Azad University, Shabestar, Iran." The authors are grateful for their financial and technical supports.

REFERENCES

[1] Barnet, E., Nordin,B. 1960. The radiological diagnosis of osteoporosis: A new approach. Clin. Radio., 11: 166-169.
[2] Duncan D. B. 1955. Multiple range and multiple ``F``test. Biometrics 11: 1-42.
[3] Fleming, R. H. 2008. National factors affecting poultry bone health. Proceedings of the Nutrition Socity. 67: 177-183.
[4] IFCC (http://ifcc.org).
[5] Jin, L. Z., Ho,Y. W. Abdullah, N., Jalaludin, S. 1997. Probiotics in poultry: Modes of actions. Words Poult. Sci. J., 53: 351-368.
[6] Katharina, E. Scholz-Ahrens, P. Ade, B. Marten, P. Weber, W. Timm, Y. Ail, C.Gluer Schrezenmeir, J. 2007. Prebiotics, probiotics and synbiotics effect mineral absorption, bone mineral content and bone structure. The J. Nut., 137: 838-846.
[7] Kwiecien, M., Winiarska-Mieczan, A. 2009. Effect of addition of herbs on body weight and assessment of physical and chemical alterations in the tibia bones of broiler chickens. J. Elementol., 14: 705-715.
[8] Liu, D., H. P. Veit, J. H. Wilson, Denbow, D. M. 2003. Maternal dietary lipids alter bone chemical composition, mechanical properties and histological characteristics of progeny of Japanese quail. Poult. Sci., 82: 463-473.
[9] Mutus,R., N. Kocabagli, M. Alp, N. Acar, M. Eren, Gezen, S. S. 2006. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. Poult. Sci., 85: 1621-1625.
[10] NRC. 1994. Nutrition requirements of poultries, National Academy Press, Washington,D. C.
[11] Osman, E. S., A. M. Abdel Maksoud, A. A. Salem, Elatar, A. H. 2009. Tibia characteristics and strength in Japanese quail fed low phosphorus diets supplemented with microbial phytase. Egypt. Poult. Sci., 29: 323-336.
[12] Reisenfeld, A. 1972. Metatarsal robusticity in bipedal rats. Am. J. Phys. Anthropol. 40: 229-234.
[13] SAS. 2001. SAS USER`s Guide: Statistic Version, SAS Institute Inc. CARY, NC, USA.
[14] Seedor, J. G., H. A. Quarruccio, Thompson, D. D. 1991. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. J. Bone Miner. Res., 6: 339-346.
[15] Talaty, P. N., M. N. Katanbaf, Hester, P. Y. 2009. Life cycle changes in bone mineralization and bone size traits of commercial broilers. Poult. Sci., 88: 1070-1077.
[16] Vahdatpour, T., H. Nikpiran, D. babazadeh, S. Vahdatpour, Jafargholipour, M. A. 2011. Effects of Protexin®, Fermacto® and combination of them on blood enzymes and performance of Japanese quails (Coturnix Japonica). Annals Biol. Res., 2: 283-291.