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Effect of *In-Ovo* Administration of L-Arginine on the Gross Anatomy of Tibia Bone, Alkaline Phospahtase and Growth Performance in Japanese Quail (*Coturnix japonica*)

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Abstract | The current study was carried out to investigate the *in-ovo* effect of L-arginine on gross anatomy of tibial bone, alkaline phosphatase enzyme and growth parameters in Japanese quail (*Coturnix japonica*). For *in-ovo* inoculation, the eggs (n = 480) were equally divided into four groups (Group I: Control, 0% L-arginine; Group II: 1% L-arginine (1 g / 100 ml); Group III: 2% L-arginine (2 g / 100 ml), and Group IV: 3% L-arginine, (3 g / 100 ml)). After hatching, the chicks of all the groups were reared on the basal diet for four weeks. Gross anatomy of tibial bone in terms of bone weight, bone length, medullary canal diameter, diaphysis, and tibio-tarsal index as well as growth performance was statistically ($p<0.05$) higher in 3% L-arginine *in-ovo* inoculated group as compared to control. Alkaline phosphatase levels were also significantly ($p<0.05$) better with 3% L-arginine *in-ovo* inoculation as compared to control. In conclusion, 3% L-arginine *in-ovo* inoculation improves morphometry of tibial bone, alkaline phosphatase levels and growth performance in Japanese quail birds.

Keywords | Growth performance, *in-ovo,* L-arginine, Alkaline phosphatase, Tibia

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INTRODUCTION

Delayed feeding just after hatching caused stunted growth, weight loss, and utilization of body reserves from the muscles, leads to poor final growth of the birds (Kornasio et al., 2011). Embryonic feeding via i*n-ovo* and feed offering just after hatching can improve the gut development, immune system, carcass quality, epigenetic and growth of the birds (Noy et al., 2010). *In-ovo* injection of commercially available diluents containing (Copper, Manganese and Zinc) has potential to significantly improve the bone mineralization in broilers (Oliveira et al., 2015).

Arginine is the limiting type of amino acids, having potential to activate the hormonal reactions in the body and act as a cell signaling molecule for the growth (Caroll et al., 2016). *In-ovo* inoculation of L-arginine significantly improved mineralization of bone and progress of growth in broilers (Sanami et al., 2014). *In-ovo* feeding of arginine significantly enhanced the gut hormones and jejunum absorptive capacity which improved the growth performance in broilers (Gao et al., 2017). Administration of amino acids via *in-ovo* in the breeder groups significantly improved the hatching percentage and weight of chicks at the time of hatching (Ohta et al., 1999). *In-ovo* inoculation of folic

acid during the incubation period significantly improved the metabolism of foliate, growth performance and epigenetic of immune genes (Li et al., 2016). Many studies are available on the *in-ovo* effects of amino acids and arginine in broilers, however there is scarcity of information on the *in-ovo* effects of arginine in quails. *In-ovo* technique is the emerging technology which meets the high demand of growing embryo during incubation period and can improve the quality of meat (Luqman et al., 2019). *In-ovo* administration of lysine amino acid can improve the immunity and histo-morphometry of thigh muscles (Luqman et al., 2020). Thus, the current study was designed with the objectives to study the *in-ovo* effects of L-arginine on the growth parameters and the morphometric characteristics of bone development in Japanese quails.

MATERIAL AND METHODS

Eggs Inoculation

All the fresh quail eggs were obtained from one breeder group, laid within 24 hour. A total number of 480 eggs were selected for *in-ovo* treatment. Group-I was serve as control (0% arginine), Group-II was given 1% L-arginine (1 g L-arginine/100 ml sterile distilled water), and Group-III was given 2% L-arginine (2 g L-arginine/100 ml sterile distilled water), Group-IV was given 3% L-arginine (3g L-arginine/100 ml sterile distilled water). By using an egg borer/egg driller a hole was made and 0.5 milliliter (ml) of the L-arginine solution was injected by 27-gauge needle of about 0.5 inch (15mm) depth into the air cell of each egg. Pyodine antiseptic was used to disinfect administered site pre and post injection, the hole was air tightly closed with hot liquid paraffin, and eggs were shifted to incubator for hatching.

Performance of Birds

Out of four eighty eggs, 240 chicks came out with 50% hatchability after completing 17th day of incubation. All the chicks were active and healthy. Before the shifting of birds to the shed, it was cleaned and fumigated. The birds were weighed and divided into four groups, 60 birds in each group. Birds were reared at experimental sheds at Avian Research and Training (ART) center, UVAS, Lahore. The experimental shed was cleaned and sterilized. The birds were maintained in sheds under standard conditions and feed intake and weight gain was evaluated on weekly basis that used to calculate FCR (feed conversion rate). At the end of experiment on 28th day, four birds per replicate were selected for slaughtering. A 2 ml blood was collected from each bird into the test tube without anticoagulant at the time of slaughtering for serum alkaline phosphatase (ALP) determination by using 23 gauge needles. These samples were send to University Diagnostic Laboratory where those were investigated according to procedures of Walter and Schutt (1974). All the experimental procedures including slaughtering of birds were done according to ethical guidelines of Ethical Review Committee of University.

Morphometric Characteristics of Bone

For bone analysis, tibia bone of birds were separated, boiled in water for ten minutes at 100 °C and then air-dried at room temperature. Weight of tibiae bones was measured by using digital weight balance and length of bones was measured with digital Vernier caliper. Outside diaphysis diameter of tibia bone was measured at the mid-point. The tibia bone medullary canal diameter (MCD) was measured by breaking the bone at mid-point and thickness of bony wall was measured by using digital Vernier caliper. Bone Tibiotarsal index was calculated by the following formula [(diaphysis diameter- medullary canal diameter)/ diaphysis dia]100.

Statistical Analysis

Statistical analyses were carried out with SPSS (Version 20). One way-ANOVA was used to analyze the data and results were presented as mean ± SEM. Tukey's test was used to compare the group differences and were considered significant at $P<0.05$.

RESULTS

The data on the effect of L-arginine on the morphometry of tibial bone, feed conversion ratio, feed efficiency and alkaline phosphatase of *in-ovo* treated groups and control group of Japanese quail is given in Table 1 and 2. Overall, gross anatomy of tibial bone in terms of bone weight, bone length, medullary canal diameter, diaphysis, and tibio-tarsal index showed highly significant improvement ($P < 0.000$) with 3% L-arginine *in-ovo* inoculation as compared to control (Table 1). Growth performance in terms of feed conversion ratio was evaluated and results showed significant improvement in L-arginine inoculated groups as compared to control group (Table 2). Alkaline phosphatase (ALP) levels were also significantly greater ($P<0.000$) with 3% L-arginine *in-ovo* inoculated group as compared to control (Table 2).

DISCUSSION

L-arginine (20 and 40 mg) were reported to enhance the bone mineralization by increasing activity of the alkaline phosphatase (ALP) on phosphorous and copper minerals of tibia bone leads to growth performance in broilers (Sanami et al., 2014). Arginine is the potent stimulator of creatine, urea and nitric oxide which are cell signaling molecules. They activate the mTOR (mammalian target of rapamycin) pathways and involve in protein synthesis (Ham et al., 2014). Cell signaling pathways like mTOR

Table 1: Effect of L-arginine on the morphometry of tibial bone in *in-ovo* treated groups and control group of Japanese quail.

Parameters	Control	1% Arginine	2% Arginine	3% Arginine	P- Value
Bone weight (mg)	541.30±1.51[c]	573.10±1.47[b]	579.80±0.67[a]	583.30±0.94[a]	0.000
Bone length (mm)	48.00±0.29[c]	54.00±0.47[b]	56.20±0.61[a]	58.00±0.71[a]	0.000
Medullary canal diameter (mm)	1.26±0.002[d]	1.31±0.003[c]	1.33±0.005[b]	1.37±0.004[a]	0.000
Diaphysis diameter (mm)	2.22±0.004[d]	2.31±0.002[c]	2.33±0.005[b]	2.35±0.004[a]	0.000
Tibio-tarsal Index	41.30±0.30[b]	41.30±0.26[b]	41.70±0.30[b]	43.60±0.37[a]	0.000

[a–d] Within the same row, different superscripts indicate significantly different means (P<0.05); Values represent the Mean ± SEM

Table 2: Effect of L-arginine on the alkaline phosphatase (ALP) and performance parameters in *in-ovo* treated groups and control group of Japanese quail.

Parameters	Control	1% Arginine	2% Arginine	3% Arginine	P- Value
ALP (IU/L)	749±2.36[d]	761±1.45[c]	788±1.85[b]	807±1.19[a]	0.000
Average feed conversion rate (FCR) of 4th week	2.66±0.01[a]	2.46±0.01[b]	2.27±0.008[c]	1.86±0.07[d]	0.000

[a–d] Within the same row, different superscripts indicate significantly different means (P<0.05); Values represent the Mean ± SEM

activated by injecting arginine in the developing embryo of human and pig which enhanced the viability of embryo (Kong et al., 2012). Delay feeding led to stunted growth resulted due to under development of gut and damage to enterocytes reported in turkeys (Potturi et al., 2005). *In-ovo* injection of hormones of growth enhanced the growth performance in meat birds (Kocamis et al., 1999). *In-ovo* feeding can activate the Satellite cell in ducks which significantly improved the diameter of muscle fiber, cross sectional area and ultimately the growth performance (Liu et al., 2012). Immune system of birds plays a vital role in providing the protection against the feed antigens especially in new born chicks, which have low immunity. *In-ovo* feeding of threonine accelerates the synthesis of immunoglobulins and mucin2 gene expression (Kermanshahi et al., 2017). Significant increase in the development of intestine and its functional capacity was observed by injecting zinc and methionine at the 17th day of incubation (Tako et al., 2005). Vitamins are the cofactors for many metabolic reactions in body, which is investigated by providing Vitamin-A and Vitamin-C via *in-ovo* (Bhanja et al., 2007). Significant effect on development of muscles and their growth was observed by feeding arginine in the feed (Fernandes et al., 2009). Relative weight of visceral organs like, proventriculus, gizzards, small intestine and liver was reported higher in those birds which are inoculated with carbohydrates (Bhanja et al., 2008). Significant improve in the immune system was investigated by injecting Vitamin-E during the incubation period (Gore and Qureshi, 1997). It is observed that development of intestinal mucosa and goblet cells occur in late stage of incubation and just after hatching, so injection of carbohydrates at that time may cause significant changes in them (Smirnov et al., 2006). Hatchability percentage and carcass quality can be improved by L-carnitine inoculation in the broiler eggs, this also affect the carcass yield and quality (Keralapurath et al., 2010). *In-ovo* inoculation affects the quality genes which cause increase in the growth performance (Liu et al., 2012). Availability of limiting nutrients in the fast growing strains of broiler can cause increase in the mortality rates, poor nutritional status at the time of birth which leads to decrease the final body weight of the birds (Ebrahimi et al., 2017). For the best results of hatching time of inoculation should be considered critically (Salahi et al., 2011). Virus and other pathogens can be deactivated by preserving the tissue sample in formalin for 24 hour which is necessary for the preparation of decontaminated tissue for histo-morphometry (Luqman et al., 2020). Significant improvement in the hatchlings was observed after the inoculation of amino acids in the yolk (Ohta and Kidd, 2001). Changes in the morphology of intestine mucosa studied up to 12 days of age after post hatch, but most observed changes in the enterocytes observed within 24 hour after hatch (Geyra et al., 2001). Developments of gut and gut associated lymphoid tissue are combined effect due to delayed in the feeding after post hatch (Shira and Friedman, 2005). Injecting the amino acid like arginine influences growing embryo, and improves the post-hatch production performance. It can also be concluded that weight loss during the transportation was due to stress; this can be overcome by the provision of arginine during embryonic development. Broiler farm economy index and broiler feed price ratio were best in those groups fed *in-ovo* (Nayak et al., 2016).

CONCLUSION

This study concludes that 3% L-arginine *in-ovo* inoculation improves the growth performance, tibia growth and alkaline phosphatase levels in Japanese quail birds compared to the control. This technique overcomes pre- and

post-hatchability nutrient and energy requirements and has greater potential in poultry industry.

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CONFLICT OF INTEREST

There exists no conflict of interest among the authors for consideration and publication of this manuscript.

AUTHORS CONTRIBUTION

Zubair Luqman, Saima Masood, Sajid Hameed, Hafsa Zaneb: Experimental Trial and Revision. Rana Waseem Akhtar, Syed Aftab Hussain Shah, Naveed Hussain, Sadaf Aslam, Nasir Iqbal: Formatting, Setting and Revision.

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