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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

In ovo injection of L-arginine on performance and bone mineralization in broiler chicken

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Abstract

This study evaluated the influence of *in ovo* injection (IOI) of various levels of L-arginine (LA) on performance and bone minerals in hatched chickens. A total of 500 fertile eggs were randomly distributed into 5 groups (100 eggs for each group). Three groups were injected with 20, 40, or 60 mg LA per egg dissolved in 0.5 ml normal saline. Rests two groups were used as un-injected control and injected control (injected with 0.5 ml normal saline). The hatched chickens from each group were randomly assigned to 4 replications of 12 chickens and reared under standard condition. A quadratic increase (P=0.007) in hatchability percentage and linear increase (P≤0.008) in body weight (BW), alkaline phosphatase (ALP) activity and tibial contents of phosphorous (P) and copper (Cu) was observed with increasing levels of LA in hatched chickens. In addition, Ca content of tibia increased (Linear: 0.001; Quadratic: 0.003) with increasing levels of LA. Body weight gain (BWG), feed intake (FI) and the activities of ALP and aspartate amino transferase (AST) linearly increased (P≤0.041) with increasing levels of LA at 21 day of age. The results indicated that LA at the level of 20 or 40 mg per egg can accelerate broiler performance and bone minerals.

Keywords: Broiler; bone; *in ovo*; L-arginine

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Introduction

Egg characteristics play vital role in influencing hatchability and growth performance during pre and post embryonic life in birds (Narushin and Romanov, 2002; Abiola et al., 2008). Nowadays, *in ovo* injection (IOI) of amino acids (AAs) is a common method to fortify breeder eggs (Foye et al., 2007; Al-Daraji et al., 2012). The embryo utilizes AAs for tissue growth at a much higher rate during incubation. At this stage, some AAs may be deficient to meet embryonic requirements (Bhanja and Mandal, 2005). It is reported that IOI of AAs mixture into broiler breeder eggs led to higher body weight at hatch and final age (Ohta et al., 1999). It is suggested that increase in weight may be due to a higher content of AAs in the yolk or the better utilization of AAs by the embryo (Ohta et al., 2001).

One of essential AAs is Arginine (Arg) which participates in protein synthesis. Chickens are exclusively depending on dietary sources for Arg (Khajali and Wideman, 2010). It is involved in a number of metabolic functions in the body, such as production of glucose (glycogenic acid), and its ability to be catabolized to produce energy (Tong and Barbul, 2004). It is utilized in a number of metabolic pathways that produce a variety of biologically active compounds, e.g. nitric oxide, creatine, agmatine, glutamate, polyamines, ornithine, and citrulline (Wu and Morris, 1998). The infusion of Arg stimulates growth hormone secretion from the anterior pituitary (Campbell et al., 2004). It also enhances the synthesis of proline and hydroxyproline, which are required for the production of connective tissue (Khajali and Wideman, 2010). A greater capacity to digest and

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absorb nutrients from an exogenous diet was obtained by IOI of Arg in turkeys (Foye et al. 2007). Therefore, the present study was conducted to test IOA of Ag supplementation on performance and bone mineralization of post-hatched chickens.

Materials and Methods

Eggs incubation and injection

Experiment was conducted utilizing 500 fertilized commercial eggs form the broiler breeders flock (Ross 308; broiler breeders age, 48 weeks, first cycle of production, average egg weight, 62.3 g, and production percentage, 71.8%). All eggs weighted and allocated to 5 treatment groups of 100 eggs each. Through IOI, LA was administered to the fertile eggs on d 1 of incubation. Treatments included un-injected control, injected control (0.5 ml normal saline), and IOI of L-arginine (LA) at the rate of 20, 40 and 60 mg per egg using 25 mm needle as standardized method (Bhanja et al., 2004). The injection site was disinfected with 70% ethanol before injection and the solutions were warmed to 30°C. Immediately after the injection, the pinhole site was sealed with sterile paraffin wax and eggs were returned to the incubator to complete the hatching process. After 18 d of incubation, the eggs were shifted to the hatching unit and kept in the respective pedigree hatching boxes. On the day of hatch, chickens were weighed and hatching percentage was recorded.

Bird management and feeding

The 1 d old chickens were evenly distributed into the same treatment groups with 4 replicates of 12 chickens per replicate. All chickens were reared under similar managerial and hygienic conditions for three weeks. The chickens were fed a basal diet containing 215.6 g CP/kg and 3,000 kcal metabolizable energy/kg to meet the nutrients requirements (NRC, 1994). The lighting schedule was 23 h at 32°C for the first day. This was subsequently reduced by 3°C each week until the end of the third week. Mash diet and fresh water were offered *ad libitum*. Weight gain (WG) and feed intake (FI) were measured cumulatively and feed conversion ratio (FCR) was calculated accordingly.

Parameters

On 1 and 21 d of age, 2 chicks were randomly selected from each pen and the blood of selected birds was taken from wing vein. After overnight clotting at 4°C, the samples were centrifuged (1,000 × g for 20 min). The separated serum was transferred to a laboratory and serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine amino-transferase (ALT) activities were measured using commercial diagnostic kits (Biosystem-EN ISO 13485, Spain). Birds were slaughtered and right tibia from each bird was collected. The right tibias were boiled for 2 min, the surrounding meat and cartilaginous caps were removed. The bones were dried in a forced-air oven for 24 h at 105°C and weighed. All tibias were ether extracted for 12 h before ashing in a muffle furnace at 480°C for 16 h. The mineral contents (Ca, P, and Cu) of the tibia bone samples were determined by ICP (Integra XL GBC, USA).

Statistical analysis

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS software (SAS, 2008). Pen was used as the experimental unit and data were analyzed as a completely randomized design by the GLM procedure of SAS software (SAS, 2008). Statistical differences were established using a Duncan's Multiple Range Test at the level of $P<0.05$. The polynomial orthogonal contrasts were carried out for LA levels to investigate the linear, quadratic, or cubic trends. The cubic trends were insignificant, thus only linear and quadratic trends were reported.

Results

Table 1 showed that quadratic increases ($P=0.007$) in hatchability percentage (68 ~ 89%) with increasing levels of LA. However, a linearly increase ($P=0.005$) was found in body weight of chickens at hatch with increasing levels of LA. A linear increase ($P\leq0.008$) was achieved in ALP activity, as well as the right tibia contents of P and Cu at hatch d with increasing levels of LA. On hatch d, Ca content of right tibias increased (Linear: 0.001; Quadratic: 0.003) with increasing levels of LA. The activities of ALP and AST was linearly increased ($P=0.012$) at 21 d of age with increasing levels of LA (Table 2). The performance of chicken is present in Table 3. A linear increase ($P\leq0.041$) in BWG and FI was attained with increasing levels LA at 1-21 d of age. No significant trends were found in other parameters.

Discussion

Increase in hatchability is in agreement with other researcher (Al-Daraji et al., 2012) who reported that IOI of LA increased the hatchability rate in Japanese quail. Quadratic increase in hatchability percentage indicates that LA at level 60 mg/egg failed to increases hatchability percentage. ALP plays an important role in ossification and calcification (Kim et al., 2008). In addition, Cu is essential mineral in synthesis of of collagen (Libby and Aikawa, 2002) and improves elasticity of bone (Gralak et al., 2004). Increases in serum ALP activity is associated with accumulation of right tibia P, Cu, and Ca contents in present study. It is

Table 1: Hatched day measured parameter affected by *in ovo* injection of L-arginine[1]

Item	Hatch (%)	BW[1] (g)	ALP[2] (U/l)	AST[3] (U/l)	ALT[4] (U/l)	DM (%)	Ca	P	Cu
Injected control	67.67^{c}	44.57^{c}	605.33^{c}	192.92	22.46	54.52	7.53^{b}	3.83^{c}	3.09^{b}
Un-injected	79.67^{ab}	45.22^{c}	628.00^{bc}	197.66	23.36	54.52	7.23^{b}	3.96^{bc}	3.24^{b}
LA, 20 mg/egg	84.33^{a}	46.85^{bc}	644.67^{b}	193.03	22.38	55.73	7.23^{b}	4.07^{abc}	3.47^{b}
LA, 40 mg/egg	88.67^{a}	50.07^{ab}	682.33^{a}	201.53	22.45	53.75	7.62^{b}	4.49^{ab}	4.19^{a}
LA, 60 mg/egg	73.00^{bc}	51.60^{a}	711.00^{a}	194.06	22.37	53.26	8.77^{a}	4.66^{a}	4.46^{a}
SEM	3.275	0.893	10.846	2.346	0.471	0.80	0.165	0.110	0.155
P-value	0.002	0.017	0.002	0.7919	0.9708	0.9245	0.005	0.043	0.003
Linear	0.25	0.005	0.003	0.93	0.59	0.57	0.001	0.008	0.001
Quadratic	0.007	0.97	0.58	0.83	0.71	0.70	0.003	0.86	0.90

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means; [1]The minerals express as mg/g ash; [1]Weight gain; [2]Alkaline phosphatase; [3]Aspartate aminotransferase; [4]Alanine aminotransferase.

Table 2: Influence of *in ovo* injection of L-arginine on measured parameter at 21 days of age[1]

Item	ALP[2] (U/l)	AST[3] (U/l)	ALT[4] (U/l)	DM (%)	Ca	P	Cu
Injected control	269.00^{b}	102.92^{b}	18.68^{ab}	44.16^{b}	14.81	7.05	0.98
Un-injected control	272.67^{b}	102.66^{b}	18.89^{ab}	50.16^{a}	15.55	7.10	1.02
LA 20 mg/egg	287.67^{ab}	102.23^{b}	18.36^{b}	50.52^{a}	15.62	7.62	1.07
LA, 40 mg/egg	293.67^{ab}	103.10^{ab}	19.10^{a}	51.65^{a}	16.68	7.97	1.12
LA, 60 mg/egg	305.00^{a}	104.205^{a}	19.14^{a}	51.79^{a}	16.70	8.02	1.17
SEM	0.10	0.029	0.035	0.016	0.51	0.17	0.45
P-value	5.001	0.221	0.098	0.919	0.380	0.165	0.034
Linear	0.012	0.001	0.10	0.37	0.29	0.034	0.16
Quadratic	0.80	0.14	0.15	0.94	0.98	0.41	0.96

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means; [1]The minerals express as mg/g ash; [2]Alkaline phosphatase; [3]Aspartate aminotransferase; [4]Alanine aminotransferase

Table 3: Effect of *in ovo* injection of L-arginine on broiler chicken performance (1-21)

Item	BWG[1] (g/d/bird)	FI[2] (g/d/bird)	FCR[3]
Sham	38.31^{b}	59.98^{b}	1.57
Un-injected	38.16^{b}	59.99^{b}	1.57
LA, 20 mg/egg	42.08^{ab}	64.67^{ab}	1.54
LA, 40 mg/egg	42.44^{ab}	67.50^{ab}	1.59
LA, 60 mg/egg	49.37^{a}	72.00^{a}	1.48
SEM	1.619	0.92	0.49
P-value	0.16	1.708	0.021
Linear	0.041	0.014	0.33
Quadratic	0.64	0.97	0.43

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means; [1]Body weight gain, [2]Feed intake, [3]Feed conversion ratio.

possible that Arg facilitates mineral uptake thereby raise the enzyme activity and help bone formation in broiler chicken. Activities of ALT and AST in serum are usually considered as an important index for understanding the liver health and activity (Pratt and Kaplan, 2000). The activities of ALT and AST increased as result of LA at 21 d of age. It may be related to hepatocytes sensitivity to growth hormone induced by Arg stimulation (Cravener et al., 1989). It is opposite to the finding of Grodzik et al. (2013) in response to IOI L-glutamine. Results illustrated that LA increased BWG and FI. This may be due to fact that Arg stimulates the secretion of the growth hormone from the anterior pituitary (Campbell et al., 2004). During embryonic growth, hepatocytes are capable of responding to the growth hormone by converting T4 to T3 and decreasing type III iodothyronine deiodinase (Darras et al., 1990 & 1992). Moreover, hepatocytes derived from chicken embryos respond to growth hormone with an increased insulin-like growth factor by continuous infusion (Cravener et al., 1989) or daily injections (Burke et al., 1987) and has beneficial effects on growth in young chicken. A broad spectrum effects exerts by the growth hormone, in turn result in somatic growth and maintenance of fuel homeostasis. Supplemental Arg resulted in major improvement in growth performance criteria (Cravener et al., 1989; Ohta et al., 2001; Flakoll et al., 2004; Uni et al., 2005; Foye et al., 2007; Al-Daraji et al., 2012). Another possible growth potential of Arg is its role in the synthesis of creatine. Arginine, glycine and methionine are the three AAs involved in the synthesis of creatine. It has been suggested that creatine increases muscular growth (Vandenberghe et al. 1997) thereby increases weight gain.

Conclusions

It can be concluded the *in ovo* injection of L-arginine at the levels of 20 or 40 mg/egg on first day of incubation could improve hatchability rate, weight gain and mineral accumulation in bone.

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