RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
PRINT ISSN 2221-1896, ONLINE ISSN 2223-0343
www.roavs.com

Influence of *in ovo* injection of L-arginine on productive and physiological performance of quail

Hazim J. Al–Daraji, A. A. Al–Mashadani, W. K. Al–Hayani, A. S. Al–Hassani and H. A. Mirza

University of Baghdad, College of Agriculture, Department of Animal Resource, Baghdad, Iraq

Abstract

This study evaluated the influence of inoculation of different levels of L–arginine into eggs of 0-day-old quail embryos. On 0 day of incubation, 480 eggs (120 for each treatment group) were injected with 0% arginine (C group); 1% arginine (T1); 2% arginine (T2); or 3% arginine (T3). After hatching, 336 quail chicks (84 chicks produced from each *in ovo* injection treatment) were placed in an experimental quail house and distributed into 4 treatment groups of 3 replicates each with 16 quail chicks for each replicate. Traits determined in this study were hatchability rate, initial body weight (7 days of age), final body weight (42 days old), feed intake, weight gain, feed conversion ratio, proportional weights of carcass, breast, legs, back bone, wings, neck, abdominal fat, liver, heart, and gizzard, blood serum glucose, protein, cholesterol, total lipids, triglycerides, calcium and phosphorus and Results revealed that *in ovo* injection with different levels of L–arginine on 0 day of incubation resulted in significant increase ($P \leq 0.05$) in hatchability rate, initial body weight, final body weight, feed conversion ratio and serum glucose, protein, total protein, calcium, phosphorus and proportional weights of carcass, breast, legs, liver, heart, and gizzard and significant decrease ($P \leq 0.05$) in serum cholesterol, total lipids, triglycerides and proportional weight of back bone, wings and abdominal fat. In conclusion, the inoculation of different levels of L–arginine into eggs of 0–day–old quail embryos especially at the levels of 2% and 3% resulted in significant improvement in productive and physiological performance of quail. Hence *in ovo* injection with L–arginine could be used as a beneficial tool for enhance productive performance of quail.

Keywords: *In ovo* Injection, Arginine, Productive Performance, Quail

Introduction

Various factors play important roles in influencing hatchability efficiency and growth performance during embryonic and post–hatch life, such as genetic, egg characteristics and incubation environment (Narushin and Romanov, 2002; Petwket et al., 2003; Abiola et al., 2008). Hatching drastically changes the way chicks retrieve nutrients. During incubation, energy and nutrients are provided via the yolk, which is rich in lipids but has relatively low protein and carbohydrate concentrations. In the embryo, yolk lipids are directly transported to the blood by endocytosis (dosSantos et al., 2010), but after hatching, yolk content is absorbed through both the yolk sac membrane and Meckel's diverticulum, and is digested and absorbed by the intestinal tract. The yolk sac energy reserves present at hatch may not be sufficient to supply their maintenance energy requirements (Dibner et al., 1998), and fasting effects may occur before the flock is removed from the hatchery. However, despite being capable of ingesting feed, the intestinal tract of the chick is still immature (Uni et al., 2003). Ohta et al. (1999) indicated that injecting an amino acid mixture into growing embryos of broiler breeder eggs resulted in higher rich body weight at 56 day of age as compared with chick from control embryos. Ohta et al. (2001) suggested that the increase in hatching weight of 7-days-old embryos injected with amino acids may have been due to a higher content of amino acids in the yolk or the better utilization of amino acids by the embryo. Foye et al. (2006) observed higher body weight, thigh weight and breast of 1-day old turkeys when these were inoculated at 23 day of incubation with egg–derived protein.

Arginine is a basic amino acid and is classified as a conditionally essential amino acid. One of the main functions of arginine is its participation in protein synthesis. Also, arginine is involved in a number of other roles in the body such is its potential to convert to glucose (hence its classification as a glycogenic acid); and its ability to be catabolised to produce energy (Tong and Barbul, 2004). Arginine is utilized by a number of metabolic pathways that produce a variety of biologically active compounds such as nitric oxide,

creatine, agmatine, glutamate, polyamines, ornithine and citrulline (Wu and Morris, 1998). Owing to the importance of the hatchability and early post–hatch growth on the market size of the birds a study was undertaken to examine the effect of *in ovo* injection of L–arginine on hatchability, productive performance, carcass traits and physiological performance of quails.

Materials and Methods

An experiment was conducted utilizing 480 fertilized commercial eggs of Japanese quail (*Coturnix coturnix japonica*). All eggs were obtained from the same breeder flock and were laid within a 24 hours period. These eggs were evenly distributed among 4 treatment groups of 120 eggs each, such that the weight distribution profile among 4 treatment groups was identical. Al-Murrani (1978) suggested that differences in protein content of eggs at days 0 and 7 of incubation affect the growth of embryos. In addition, Vitamin D precursor administration into eggs has been done from day 0 of incubation in Japanese quail (ELaroussi et al., 1993). If amino acid administration is possible before incubation, it may be safer. Therefore, arginine was administered on day 0 of incubation. A hole was incised using automatic needle and 0.5 ml of L–arginine solution was injected into the air cell using a 23–gauge needle to a depth of about 15 mm. The injection site was disinfected with 70% ethanol before and after injection, sealed with nail paint and transferred to incubating baskets. The *in ovo* injection solutions were: 0% arginine (just distilled water; control group); 1% arginine (1 g arginine/100 ml distilled water; T1); 2% arginine (2 g arginine/100 ml distilled water; T2); 3% arginine (3 g arginine/100 ml distilled water; T3). After injection, eggs were transferred to incubator to complete hatching process.

After 18 days of incubation, hatched quail chicks were removed from the hatcher to determine hatchability rate. Chicks were then transported to the experimental house. The day-old chicks (336chicks; 84 chicks from each *in ovo* injection treatment) were evenly distributed into the same treatment groups (4 *in ovo* injection treatments) with 3 replicates per group and each replicate contain 28 chicks. For 6 weeks (whole period of experiment) the quail were fed diet containing 21% protein and 2888 Kcal/kg metabolizable energy. The quail were allowed free access to food and water and housed in wire cages with 28 quail for each cage. A regime of 17 hours constant lighting and continuous ventilation were provided and all birds were kept under uniform management conditions throughout the experimental period.

Productive traits included in this study were initial body weight (7 days old), final body weight (42 days of age), feed intake, body weight gain, and feed conversion ratio. The later 3 traits were determined on a weekly basis and the data exhibited as a total mean for the whole period of experiment (6 weeks).

At the end of experiment blood samples were collected from 18 birds in each treatment (6 birds from each replicate). Blood were collected from jugular vein (Al–Daraji et al., 2008) to ensure a free flow of blood. Blood samples were used after pooled blood samples for each replicate in treatment group to evaluate serum chemistry traits. After overnight clotting at 4 °C, the samples were centrifuged for 20 min at 4,000 x g. The separated serum was transferred to a private laboratory and was analyzed for glucose, total protein, cholesterol, total lipids, triglycerides, calcium and phosphorus. Blood traits included in this study were analyzed by using standard methods reported by Al – Daraji et al. (2008).

On the last day of experiment, feed was withheld of all birds overnight to facilitate gut clearance. Birds were weighed to obtain live body weight. After recording the weights of birds they were slaughtered, defeathered and processed (removal of gastrointestinal tract). Carcass was stored at 4°C for 24 hours. Afterward, the weights of carcasses were recorded, the carcasses were dissected to determine the percentage of carcass morphology by measuring the weights of liver, heart, gizzard, breast, legs, back bone, wings, neck and abdominal fat.

Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to GLM procedure of SAS software (SAS, 2000). The significant differences among means were determined by using Duncan's multiple range tests. Differences among treatment means were compared at $P \leq 0.05$.

Results

The effect of *in ovo* injection with different level of arginine at 0 day of age on hatchability rate, initial body weight (7 days of age), final body weight (42 days of age), feed intake, weight gain and feed conversion from hatching to 6–week–old of quail are shown in Table 1. *In ovo* injection with 1%, 2% or 3% arginine increased ($P \leq 0.05$) hatchability rate compared with control. However, there was no significant difference between T2 and T3. Results revealed that chicks produced from eggs injected with L–arginine especially at level of 2% and 3% were heavier ($P \leq 0.05$) in body weight at 7–day–old and 6–week of age from hatch to 6 weeks of age than those produced from control eggs. The best results of feed conversion during 0–6 weeks of age were realized from chicks hatched from eggs injected with L–arginine ($P \leq 0.05$) compared with those hatched from control eggs. Treatment had no significant effect on feed intake through 0–6 weeks of age. Furthermore, there were no significant differences between T2 and

Table 1: Effect of *in ovo* injection of L – arginine on hatchability rate and productive performance of quail (means ± S.E)

Traits	Treatments			
	C	T1	T2	T3
Hatchability (%)	81.31 ± 2.23[c]	86.93 ± 1.65[b]	90.24 ± 3.31[a]	91.45 ± 2.77[a]
Initial body weight (7 days) (g)	19.08 ± 0.80[c]	20.92 ± 0.76[b]	22.61 ± 0.23[a]	23.09 ± 0.27[a]
Final body weight (42 days) (g)	221.32 ± 13.50[c]	233.61 ± 11.25[b]	240.92 ± 16.25[a]	246.33 ± 19.33[a]
Feed intake (g)	790.12 ± 55.20	792.32 ± 60.02	795.01 ± 67.13	799.01 ± 71.38
Body weight gain (g)	195.26 ± 22.19[c]	200.41 ± 30.11[b]	210.91 ± 29.91[a]	217.87 ± 20.92[a]
Feed conversion ratio	4.04 ± 0.09[a]	3.95 ± 0.04[b]	3.76 ± 0.06[c]	3.66 ± 0.02[d]

[a – d]Means within rows with different superscripts differ significantly at P≤ 0.05; C: Control group; T1, T2, and T3: represent *in ovo* injection of 1%, 2%, 3% L–arginine, respectively.

T3 concerning initial body weight, final body weight and weight gain, while T3 group excel other treatments with relation to feed conversion. Serum glucose, total protein, cholesterol, total lipids, triglycerides, calcium and phosphorus of chicks at 6 – weeks of age differ significantly (P≤0.05) under different treatments (Table 2). Chicks produced from eggs injected with L arginine showed significant increase (P≤0.05) in serum glucose, protein, calcium and phosphorus and significant decrease (P≤0.05) in serum cholesterol, total lipids and triglycerides compared to those hatched from control eggs. On the other hand, there were no significant differences between T2 and T3 in respect to all blood serum traits included in this study.

Table 3 showed significant (P≤0.05) differences between treatments regarding carcass traits and relative weight of certain organs. The overall means of carcass weight (%) and the proportional weights of breast, legs, liver, heart, and gizzard were higher (P≤0.05) in chicks hatched from eggs injected with L–arginine as compared with chicks produced from control group. Moreover, chicks produced from eggs inoculated with L – arginine recorded the lowest relative weights of back bone, wing, neck and abdominal fat compared to those hatched from control eggs. However, there were no significant differences between T2 and T3 with respect to carcass characteristics and organs weight.

Discussion

The best results obtained in this study when quail eggs injected with L–arginine regarding carcass traits and blood serum metabolites may be explained by the way arginine stimulates the secretion of growth hormone. Campbell et al. (2004) reported that the infusion of arginine stimulates growth hormone secretion from the anterior pituitary. This increase in growth hormone secretion from arginine infusion has been attributed to the suppression of endogenous somatostatin secretion (Alba– Roth et al., 1988).

Darras et al. (1990; 1992) indicated that during embryonic growth, liver cells are capable of responding to growth hormone by converting T4 to T3 and decreasing type III iodothyronine deiodinase. Moreover, hepatocytes derived from chick embryos respond to growth hormone with increased insulin–like growth factor by either continuous infusion or daily injections, has significant effect on growth and carcass composition in young chicken (Burke et al., 1987; Cravener et al., 1989). Growth hormone exerts a broad spectrum of effects which result in somatic growth and maintenance of fuel homeostasis. The effects are diverse and include reduction in lipid synthesis, enhance growth and protein synthesis, alterations of carbohydrate metabolism, increase the levels of calcium, phosphorus, protein, glucose in blood, stimulate erythrocyte synthesis and cellular differentiations (Harvey and Etches, 1997). Chevalley et al. (1998) and Flakoll et al. (2004) reported that arginine supplementation resulted in significant improvement in growth criteria and physiological performance. The *in ovo* fed avian neonate may have a greater capacity to digest and absorb nutrients from an exogenous diet relative to the conventional hatching (Foey et al., 2007). Previous *in ovo* feeding experiments (Tako et al., 2004) demonstrated that *in ovo* injection of leucin metabolite, β–hydroxyl–β–methyl–butyrate had a 45% increase in jejunal villus surface area at hatch in comparison with the controls. Another possible growth potential of arginine is its role in the synthesis of creatine. Arginine, glycine and methionine are the three amino acids involved in the synthesis of creatine. Creatine has been shown to increase muscular growth and strength (Vandenberghe et al., 1997) and muscle fiber size (Volek et al., 1999). On the other hand, arginine is a precursor of the cell–signing molecule nitric oxide. Nitric oxide acts as signaling molecule facilitates the dilation of blood vessel and decrease vascular resistance. Nitric oxide is synthesized from arginine under the enzymatic control of nitric oxide synthase. Muscle growth and functions regulated by nitric oxide or related molecules include force production (excitation – contraction coupling), auto regulation of blood flow, myocyte differentiation, respiration and glucose homeostasis (Stamler and

Table 2: Effect of *in ovo* injection of L – arginine on some blood serum traits of quail (means ± S.E)

Traits	Treatments			
	C	T1	T2	T3
Glucose (mg/100 ml)	253.11 ± 24.08[c]	276.31 ± 18.09[b]	293.26 ± 33.15[a]	299.10 ± 20.19[a]
Total protein (g / 100 ml)	2.91 ± 0.42[c]	3.28 ± 0.61[b]	3.93 ± 0.56[a]	4.11 ± 0.36[a]
Cholesterol (mg / 100 ml)	210.22 ± 7.19[a]	183.10 ± 9.15[b]	171.22 ± 8.19[c]	168.89 ± 9.75[c]
Total lipids (mg / 100 ml)	695.13 ± 29.18[a]	583.27 ± 36.95[b]	461.96 ± 40.01[c]	452.76 ± 39.95[c]
Triglycerides (mg / 100 ml)	190.25 ± 9.92[a]	178.11 ± 18.27[b]	150.22 ± 16.91[c]	140.92 ± 17.25[c]
Calcium (mg / 100 ml)	6.03 ± 0.92[c]	7.76 ± 0.90[b]	8.65 ± 0.72[a]	8.72 ± 0.48[a]
Phosphorus (mg / 100 ml)	3.72 ± 0.17[c]	4.83 ± 0.35[b]	5.60 ± 0.29[a]	5.67 ± 0.33[a]

[a–c]Means within rows with different superscripts differ significantly at P≤0.05; C: Control group; T1, T2, and T3: represent *in ovo* injection of 1%, 2%a nd 3% L–arginine, respectively.

Table 3: Effect of *in ovo* injection of L–arginine on carcass traits and proportional weight of certain organs of quail (means ± S.E)

Traits	Treatments			
	C	T1	T2	T3
Carcass (%)	69.55 ± 2.63[c]	71.31 ± 1.77[b]	72.99 ± 3.03[a]	73.09 ± 1.23[a]
Breast (%)	34.23 ± 1.79[c]	36.07 ± 2.25[c]	37.24 ± 1.20[a]	37.50 ± 1.08[a]
Legs (%)	24.21 ± 1.33[c]	25.92 ± 2.36[b]	26.94 ± 2.08[a]	26.99 ± 1.97[a]
Back bone (%)	23.01 ± 2.29[a]	22.10 ± 1.06[b]	21.00 ± 0.98[c]	21.03 ± 1.02[c]
Wings (%)	7.86 ± 0.92[a]	6.40 ± 0.31[b]	6.03 ± 0.22[c]	5.89 ± 0.11[c]
Neck (%)	7.93 ± 0.88[a]	7.58 ± 0.76[b]	7.55 ± 0.63[b]	7.50 ± 0.80[b]
Abdominal fat (%)	2.76 ± 0.17[a]	1.93 ± 0.20[b]	1.24 ± 0.11[c]	1.09 ± 1.01[c]
Liver (%)	1.45 ± 0.22[c]	1.73 ± 0.39[c]	1.95 ± 0.17[a]	2.02 ± 0.12[a]
Heart (%)	0.85 ± 0.08[c]	0.93.00 ± 0.09[c]	1.04 ± 0.11[a]	1.07 ± 0.09[a]
Gizzard (%)	1.38 ± 0.09[c]	1.56 ± 0.08[b]	1.72 ± 0.04[a]	1.81 ± 0.06[a]

[a–c]Means within rows with different superscripts differ significantly at P≤0.05; C: Control group; T1, T2, and T3: represent *in ovo* injection of 1%, 2% and 3% L–arginine respectively; [a–c]Means within rows with different superscripts differ significantly at $p \leq 0.05$.

Meissner, 2001). Contractile activity and muscle growth greatly increase nitric oxide production in the muscle, and this is likely due to elevated intracellular calcium (Kobzik et al., 1994).

Conclusion

It may be concluded from this study that *in ovo* injection of L–arginine especially at the levels of 2% and 4% on 0 day of incubation would improve hatchability rate, productive performance, carcass traits and certain blood serum characteristics. Therefore, *in ovo* injection of L–arginine at 0 day old could be used as an efficient tool for improved productive performance of quail.

References

Abiola, S.S., Meshioye, O.O., Oyerinde, B.O. and Bamgbose, M.A. 2008. Effect of egg size on hatchability of broiler chicks. *Archivos Zootechecnia*, 57: 83–86.

Alba–Roth, J., Muller, O.A. and Schophol, J. 1988. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *Journal of Clinical Endocrinology and Metabolism*, 67 (6): 1186 – 1189.

Al Daraji, H.J., Al–Hayani, W.K. and Al–Hassani, A.S. 2008. Avian Hematology. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.

Al–Murrani, W.K. 1978. Maternal effects on embryonic and postembryonic growth in poultry. *British Poultry Science*, 19: 277–281.

Burke, W.H., Moore, J.A., Ogez, J.R. and Builder, S.E. 1987. The properties of recombinant chicken growth hormone and its effect on growth, body composition, feed efficiency, and other factors in broiler chickens. *Endocrinology*, 120: 651–658.

Campbell, B.I., LaBounty, P.M. and Roberts, M. 2004. The ergogenic potential of arginine. *Journal of Inter Society of Sport Nutrition*, 1(2): 35-38.

Chevalley, T., Rizzoli, R. Mannen, D., Caverzasio, J. and Bonjour, J.P. 1998. Arginine increases insulin–like growth factor–I production and collagen synthesis in osteoblast–like cells. *Bone*, 23:103 – 109.

Cravener, T.L., Vasilatos–Younken, R. and Wellenreitter, R.H. 1989. Effect of subcutaneous

infusion of pituitary–derived chicken growth hormone on growth performance of broiler pullets. *Poultry Science*, 68:1133–1140.

Darras, V.M., Huybrchts, L.M., Berghman, L., Kuhn, E.R. and Decuypere, E. 1990. Ontogeny of the effect of purified chicken growth hormone on the liver 5' monodeiodination activity in the chicken: reversal of the activity after hatching. *General and Comparative Endocrinology*, 77, 212 – 220.

Darras, V.M., Berghman, L.R., Vanderpooten and Kuhn, E.R. 1992. Growth hormone actually decreases type III iodothyronine deiodinase in chichen liver. *FEBS Letters*, 310: 5–8.

Dibner, J.J., Knight, C.D., Kitchell, M.L., Atwell, C.A., Downs, A.C. and Ivey, F.J. 1998. Early feeding and development of the immune system in neonatal poultry. *Journal of Applied Poultry Research*, 7: 425-436.

dos Santos, T.T., Corzo, A., Kidd, M.T., McDaniel, C.D., Torres Filho, R. A. and Araujo, L.F. 2010. Influence of in ovo inoculation with various nutrients and egg size on broiler performance. *Journal of Applied Poultry Research*, 19: 1–12.

Elaroussi, M.A., Deluca, H.F., Forte, L.R. and Biellier, H.V. 1993. Survival of vitamin D–deficient embryos: Time and choice of cholecalciferol or metabolites for treatment *in ovo*. *Poultry Science*, 72: 1118–1126.

Foye, O.T., Ferket, P.R. and Uni, Z. 2007. The effects of *in ovo* feeding arginine, β–hydroxyl–β–Methyl – Butyrate, and protein on jejunal digestive and absorptive activity in embryonic and neonatal turkey poults. *Poultry Science*, 86, 2343 – 2349.

Foye, O. T., Uni, Z. and Ferket, P. R. 2006. Effect of in ovo feeding egg white protein, β–hydroxyl –β–methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. *Poultry Science*, 85:1185–1192.

Flakoll, P., Sharp, R., Baier, S., Levenhagen, D., Carr, C. and Nissen, S. 2004. Effect of β–hydroxyl–β–methylbutyrate, arginine, & lysine supplementation on strength, functionality, body composition, and protein metabolism. *Nutrition*, 20, 444 – 451.

Harvey, S. and Etches, R.J. 1997. Perspectives in Avian Endocrinology. J. W. Arrowsmith Ltd, Bristol, UK.

Kobzik, L., Reid, M. B., Bredt, D. S. and Stamler, J.S. 1994. Nitric– oxide in skeletal muscle. *Nature*, 372: 546–548.

Narushin, V.G. and Romanov, M.N. 2002. Egg physical characteristics and hatchability. *World's Poultry Science*, 58: 297–302.

Ohta, Y., Kidd, M.T. and Ishibashi, T. 2001. Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after *in ovo* administration of amino acids. *Poultry Science*, 80:1430–1436.

Ohta, Y., Tsushima, N., Koide, K., Kidd, M.T. and Ishibashi, T. 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poultry Science*, 78: 1493-1498.

O' Neill, I.E., Houston, B. and Goddard, C. 1990. Stimulation of insulin – like growth factor I production in primary cultures of chicken hepatocytes by chicken growth hormone. *Molecular Cellular Endocrinology*, 70:41–47.

Petwk, M., Baspinar, H. and Ogan, M. 2003. Effects of egg weight and length of storage on hatchability and subsequent growth performance of quail. *South African Journal of Animal Science*, 33: 266–273.

SAS, 2000. SAS / STAT User's Guide, Version, 6.12. Inst. Inc., Cary, NC.

Stamler, J.S. and Meissner, G. 2001. Physiology of nitric oxide in skeletal muscle. *Physiological Review*, 81 (1): 209–237.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd (Ed.) McGraw–Hillbook Co., NY.

Tako, E., Ferket, P.R. and Uni, Z. 2004. Effects of *in ovo* feeding of carbohydrates and β–hydroxyl–β–methylbutyrate on the development of chicken intestine. *Poultry Science*, 83: 2023–2028.

Tong, B.C. and Barbul, A. 2004. Cellular and physiological effects of arginine. *Mini Reviews in Medical Chemistry*, 4 (8): 823–832.

Uni, Z., Tako, E., Gal–Garber, O. and Sklan, D. 2003. Morphological, molecular and functional changes in the chicken small intestine of the late–term embryo. *Poultry Science*, 82: 1747–1754.

Vandenberghe, K., Goris, M., Van Hecke, P. and Van Leemputte. 1997. Long–term creatine intake is beneficial to muscle performance and pharmacokinetic considerations. *Journal of Applied Physiology*, 83: 2055 – 2063.

Volek, J.S., Duncan, N.D., Mazzetti, S.A. and Putukian, M. 1999. Performance and muscle fiber adaptations to certain supplementation and heavy resistance training. *Medicine & Science in Sports and Exercise*, 31:1147–1156.

Wu, G. and Morris, S.M. 1998. Arginine metabolism: nitric oxide and beyond. *Biochemistry Journal*, 336: 1–17.