



INFLUENCE OF IN OVO INJECTION OF L-ARGININE ON HATCHABILITY AND SOME PHYSIOLOGICAL TRAITS OF INSHAS LOCAL BREED CHICKEN

Abeir, A.M. Eshera, Shamiah, Sh.M.; Ragaa E. Abd El-Karim

Anim.Prod. Res. Inst., Agric. Res. Center, Mini. of Agric., Dokki, Egypt

Corresponding author: Abeir Eshera; E-mail: abeer1_ahmed1@yahoo.com

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ABSTRACT: This study was carried out to evaluate the effect of in ovo injection of different L-arginine (LA) levels (0, 1, 2 and 3%) at day 7 of incubation period on hatchability (%) and some physiological traits of Inshas local breed chickens. Fertile eggs (n=500) from Inshas laying hens were divided into 5 groups (100 eggs in each treatment group) as follow: the first and the second groups were the negative control non- injected group (G1) and the positive control (injected with saline solution) groups (G2); while the 3rd, 4th and 5th groups were injected in ovo with different L-arginine (LA) levels (1, 2 and 3%). The criteria of response were on hatchability, blood hematology and biochemical traits, as well as histological changes in duodenum, in terms of villi height, width and surface area.

Results revealed a significant ($P<0.05$) increase of hatchability rate in treated groups (G3, G4 and G5) than the control groups (G1 and G2). However, G3 group has was significantly ($P<0.05$) the lowest in percentage of hatchability (82.8 %) than in G5 (86.6%), but no significant differences between G4 and G3 and G5 were recorded. The mean values of residual Yolk % and liver (g), were significantly ($P<0.05$) higher in treated groups G5, G4 and G3 than in control groups.

In ovo injection with L-arginine, resulted in a significant ($P<0.05$) increase in hemoglobin, total white and red blood cells count in treatment groups than control groups. Moreover, PCV (%) and Heterophils (%) were significantly higher in groups G4 and G5 than in groups G1 and G2 but no significant difference among G3 and other groups. On the other hand, lymphocytes % was not significantly affected by in ovo injection with L-arginine among groups. Chicks produced from eggs injected with L-arginine in groups G4 and G5 showed significant increases ($P>0.05$) in serum glucose and protein concentrations than in other groups and significant decreases ($P>0.05$) in serum cholesterol, total lipids and triglycerides concentrations compared with G3 and controls. Average of villus length and surface area were significantly higher in groups 5 and 4 than in G3, G2 and G1 groups, respectively. While, duodenum villus width was significantly higher in groups 5, 4 and 3 than in groups 2 and 1.

Conclusion: It can be concluded that the in ovo injection of L-arginine the 7th d of incubation would improve hatchability percentage rate, some organ weights, carcass traits and certain blood serum characteristics and hematology, as well as, villus length, width and surface area.

Keyword: In ovo injection- hatchability-L-arginine, chicken

INTRODUCTION

Many 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).

Because the difficult to control egg composition via hen nutrition (breeder feed), in ovo feeding (direct application into the egg) offers a promising solution to provide developing embryos with the essential nutrients (Uni and Ferket, 2003). The ability to directly supply growing embryos with in ovo injection (IOI) technology with specific nutrient compounds may decrease the need for long-term formulation of enriched rations for maternal diets to achieve similar effect, also provide a more accurate dose at the specific time for peak absorption of specific nutrients, cofactors by the embryo (Surai et al., 1999).

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. Uni et al., (2003) reported that, broiler breeder eggs contain an excess of fat and moisture but not protein (Al-Murrani, 1978).

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. Also, Al-Murrani (1982) demonstrated that injecting an amino acid (AA) mixture (identical to the AA pattern of egg protein) into growing embryos of broiler breeder eggs resulted in higher chick BW at hatch and at 56 d of age as

compared with chicks from control embryos.

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.

In ovo injection 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 tissues 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).

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). Al-Murrani (1978) suggested that differences in protein content of eggs at days 0 and 7 of incubation effect on the growth of embryos.

Therefore, the present study was conducted to evaluate in ovo injection of L-arginine (LA) levels (0, 1, 2 and 3%) on hatchability and physiological traits of local breed chicken (Inshas).

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MATERIALS AND METHODS

This study was carried out at the Experimental Research Station, Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Experimental design:

A total of 500 fertile eggs were collected from Inshas laying hens at 40 weeks of age, during the first cycle of production. Eggs were stored for 7 days (egg collection period) before incubation. These eggs were divided into 5 groups (100 eggs in each treatment group) as follow:

- 1- A negative control group (un-injected ; G1).
- 2- A positive control group (injected with saline solution (G2).
- 3- Injected with 1% arginine (1 g arginine/100 ml saline; G3).
- 4- Injected with 2% arginine (2 g arginine/100 ml saline; G4).
- 5- Injected with 3% arginine (3 g arginine/100 ml saline; G5).

Through in ovo injection (IOI) L-arginine was administered on day 7 of incubation. A hole was incised using automatic needle and 0.5 ml of L-arginine solutions were warmed to 30°C and 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, 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 complete the incubation period, the hatchability percentage, dead1 and dead 2 were recorded in day of hatching.

At day of hatching 6 chicks were selected randomly in each group, weighed individually and after slaughter of the residual yolk sac, liver, gizzard, heart, and spleen were weighted and recorded, as well as collection of blood samples with EDTA for blood hematology and without EDTA for blood biochemistry.

Blood samples

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 4000 x g. The separated serum was transferred to a private laboratory and was analyzed for total protein, albumin, glucose, total cholesterol, total lipids, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using commercial diagnostic kits.

Histometric measurements:

At day of hatching, samples were taken from the median part of duodenum for histological examinations. Representative samples were fixed in Bouin's solution (24 h), washed, dehydrated in ascending grades of ethyl alcohol, cleared in zylol and embedded in paraffin wax. Thereafter, the samples were sectioned at 5 microns, stained by hematoxylin and eosin stains (H&E) and histologically examined using the routine method after Bancroft et al., (1996).

Measurement of villus height and villus surface area:

The villus height, villus width and villus surface area of duodenum using nanometer eye lens. Villus height and width data were used to calculate villus surface area [$2\pi \times (W/2) \times L$], where W= villus width and L= villus length (Sohail et al., 2012).

Statistical analysis

These data were subjected to one way ANOVA using SAS software (SAS, 2001). A significant difference was used at 0.05 probability level and differences among treatments were tested using the Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISSECTION

The effect of different levels of L-arginine in ovo administrated at day 7 of incubation on hatchability rate for local breed chickens was presented in table 1. In The present study showed a significant ($P<0.05$) increase of hatchability rate in treated groups (G3, G4 and G5) than in control groups(G1 and G2). However G3 was has significantly ($P<0.05$) the lowest in percentage of hatchability (82.8 %) than in G5 (86.6%), but no significant differences between G4 and G3 and G5. Data showed also that mortality rate 1 and 2 was significantly lower in G5 than other groups. But significantly higher in G2 (12.3) and mortality rate 2 in G3 (8.9%).

The results of this study are in accordance with the reports of Al-Daraji et al.,(2012) who reported that in ovo 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 and Nouri Sanami et al., (2014) who 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.

Al-Daraji et al., (2012) found that In ovo injection with 1%, 2% or 3% L-arginine increased ($P>0.05$) the hatchability rate compared with the control. However, there was no significant difference in hatchability rate between 1% and 2%.

Because fat and moisture, but not protein, are in excess (Al-Murrani, 1978), embryonic and postembryonic growth may be improved by AA injection into the egg (Al-Murrani, 1982). 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).

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).

Data in Table 2. revealed that significant ($P<0.05$) affected in ovo injection different level of L-arginine on Yolk % and liver (g), being in groups G5, G4 and G3 than in control groups. However no significant differ among groups in live body weight (g), gizzard (g), heart (g), and spleen (g) table 2.

One the other hand, increased the main values of live body weight (g), gizzard (g) and heart (g) were by increasing level of L-arginine without significant.

Supplemental Arg resulted in major improvement in growth performance criteria (Cravener et al., 1989; Ohta et al.,

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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 (Vandenbergh et al. 1997) thereby increases weight gain.

Present results in Table (2) were agreement with Edwards et al., (2016) reported that the increase in gizzard and liver weight at hatch produced by arginine injection at day zero into the albumen, may also lead to improved efficiency of the chickens post hatch. If the liver weight increase is due to increased glycogen stores this would be especially beneficial for chicks that spend time fasting post hatch before given access to feed.

Enhanced liver development and possible storage of energy substrates may reduce the impact of this restricted period Edwards et al., (2016).

Results of Table (3) indicated that blood hematological traits of Inshas chicks were significantly affected by in ovo injection with L-arginine, resulted in a significant increase in hemoglobin, total white and red blood cells count in treatments groups than control groups. Whoever PCV (%) and Heterophils (%) were significantly higher in groups G4 and G5 than in groups G1 and G2 but no significant among G3 and other groups. while lymphocytes (%) was not significantly affected by in ovo injection with L-arginine among groups. Nevertheless H/L ratio was significantly higher in groups

G4 and G5 than control groups while G3 was not significant with all groups. In general in all parameter of blood hematology no significant differ among treatments groups. The lowest values were recorded with negative control followed by positive control for all traits except lymphocyte.

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.

Campbell et al., (2004) reported that it is well documented that the infusion of arginine stimulates growth hormone secretion from the anterior pituitary. This increase in growth hormone secretion 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 the growth hormone by converting T4 to T3 and decreasing type III iodothyronine deiodinase.

The growth hormone exerts a broad spectrum of effects, which result in somatic growth and maintenance of fuel homeostasis. These effects include reduction in lipid synthesis, enhanced growth and protein synthesis, alterations of carbohydrate metabolism, increased levels of calcium, phosphorus, protein,

glucose in blood, stimulated erythrocyte synthesis and cellular differentiations (Harvey and Etches, 1997).

Chicks produced from eggs injected with L-arginine in groups G4 and G5 showed significant increases ($P>0.05$) in serum glucose and protein concentrations than in other groups and significant decreases ($P>0.05$) in serum cholesterol, total lipids and triglyceride concentrations compared with those hatched from eggs in G3 and controls table 4. Whoever albumin and globulin concentrations in serum were significantly higher in chicks produced from eggs injected with L-arginine in groups G4 and G5 as compared to controls groups, while no significant differences between chicks in G3 and other groups. On the other hand, there were no significant differences between treatment for AL/GL ratio.

This result is in harmony with finding of Al-Daraji et al., (2012) who reported that in ovo injection with L-arginine showed significant increases ($P>0.05$) in serum glucose, protein, calcium and phosphorus concentrations and significant decreases ($P>0.05$) in serum cholesterol, total lipids and triglyceride concentrations compared with those hatched from control eggs. On the other hand, there were no significant differences between T2 and T3 for all blood serum traits included in this study.

The AA pattern of egg was constant throughout incubation. The AA of albumen are transferred to the embryo at the same ratio, regardless of incubation time (Rupe and Farmer, 1955).

Albumen is absorbed into the yolk sac (Freeman and Vince, 1974), and AA in yolk and albumen may be utilized at a constant ratio. All AA contents of eggs or embryos, except Gly and Pro, changed similarly with advancing incubation time. This relationship suggests that the AA

pattern of egg is an ideal pattern for embryonic growth.

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).

Data in table 5. revealed that activity of AST and ALT in blood serum were not significantly ($P<0.05$) affected by in ovo injection of L- Arginine. While concentration of ALP in blood serum were significantly ($P<0.05$) higher in groups G4 and G5 than in other groups Nouri Sanami et al., (2014) A linear increase ($P\leq 0.008$) 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.

Nouri Sanami et al., (2014) the activities of ALP and AST was linearly increased ($P=0.012$) at 21 d of age with increasing levels of LA. A linear increase ($P\leq 0.041$) in BWG and FI was attained with increasing levels LA at 1-21 d of age.

ALP plays an important role in ossification and calcification (Kim et al., 2008). In addition, Cu is essential mineral in synthesis of collagen (Libby and Aikawa, 2002) and improves elasticity of bone (Gralak et al., 2004). Increases in serum ALP activity is associated with

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accumulation of right tibia P, Cu, and Ca contents in present study. It is 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).

Macroscopically evaluating the small intestines, the effect of in ovo injection with L- arginine on duodenum villus length, width and surface area was presented in table 5, being that duodenum villus length, width and surface area was significant ($P>0.05$) influenced by L- arginine.

Results in table 6 indicated that duodenum villus length and surface area were significantly higher in groups 5 and 4 than in groups 3, 2 and 1. While duodenum villus width was significantly higher in groups 5, 4 and 3 than in groups 2 and 1. on the other hand, in treatment groups the values of duodenum villus length, width and surface area increased with elevated the level of L- arginine.

In ovo L- arginine injection at the 7th day of incubation improved intestine morphology including villus height, width and surface area Based on the results, the best improving effect was observed in 3 % L- arginine in ovo feeding of L- arginine. In an experiment, in ovo injection of 25 mg L-lysine at the 14th day of incubation, numerically

improved relative small intestine weight of hatchlings than control chicks (Bhanja et al., 2012). While, Bhanja and Mandal (2005) showed no differences in digestive organ weights at 21 d between in ovo injected chickens with Lys+Arg or Lys+Met+Cys and uninjected control chickens. Also, Ebrahimi et al., (2017) found that positive effects of feeding amino acid (lysine) on intestine morphology during growth period. Vaezi et al. (2011) indicated that feeding 14 g Lys/kg diet increased villus height and crypt depth which was similar to the results of the present study.

Results of different experiments showed that in ovo feeding of nutrients can solely accelerate early enteric development and function in hatchlings and then, cause higher rate of digestion and absorption during growth period (Bartell and Batal 2007; Chen et al., 2009; Uni and Ferket 2004 and Uni et al.,1998). Then, in ovo feeding of L- arginine as one nutrient was able to directly improve intestine morphology.

Observing the best improving effect of 3 % L- arginine in ovo feeding may indicate the role of this level of L- arginine in making the best amino acid balance for promoting growth of fetus.

Previous in ovo feeding experiments (Tako et al., 2004) demonstrated that in ovo injection of the leucin metabolite β -hydroxyl- β - methyl-butyrate had a 45% increase in jejunal villus surface area at hatch in comparison with the controls.

These results in the present study are in agreement with Edwards et al., (2016) who found that increased weight, length and villi number of intestinal tracts of

chickens injected in ovo at day nine of development has significant potential impact on subsequent chicken growth and efficiency. Increased development of the intestines presumably results from the known impacts of arginine on growth-regulatory pathways (Chevally et al., 1998), with IGF-1 and Growth Hormone strong candidates through their known effects on cell proliferation (Isidori et al., 1981).

CONCLUSION

It can be concluded from this study that the in ovo injection of L-arginine especially at the levels of 1%, 2% and 3% on 7 d of incubation would improve hatchability rate, some organ weights, carcass traits and certain blood serum characteristics and hematology as well as villus length, width and surface area . Therefore, in ovo injection of L-arginine at 7 day old could be used as an efficient tool to improve the productive performance of Inshas chicken.

Table (1): Hatchability (%) of Inshas local chickens as affected by in ovo injection with different levels of L–arginine.

Items	Treatment groups					SEM	Sig. test
	G1	G2	G3	G4	G5		
Egg weight (g)	43.4	43.6	43.6	43.2	43.5	±0.17	NS
Hatchability %	81.0 ^c	79.4 ^c	82.8 ^b	85.0 ^{ab}	86.6 ^a	±1.02	*
Dead 1 %	12.1	11.5	12.3	11.7	10.9	±0.84	NS
Dead 2 %	6.3 ^b	8.9 ^a	4.4 ^{bc}	3.0 ^{bc}	2.4 ^c	±0.47	*

a, b and c Means denoted within the same raw with different superscripts are significantly different at P<0.05.

NS: Not significant

Dead1 and dead2 (%) were recorded in day of hatching.

Table (2): Body and relative organs weight of Inshas local chickens as affected by in ovo injection with different levels of L–arginine.

Items	Treatment groups					SEM	Sig. test
	G1	G2	G3	G4	G5		
Pre slaughter live body weight (g)	33.2	32.9	33.9	35.3	36.8	±0.61	NS
Yolk sac (g)	4.00 ^a	4.07 ^a	3.23 ^b	3.00 ^b	2.80 ^b	±0.15	*
Liver (g)	3.16 ^c	3.17 ^c	3.36 ^{bc}	3.73 ^b	4.28 ^a	±0.13	*
Gizzard (g)	5.61	5.66	5.57	5.54	5.71	±0.21	NS
Heart (g)	0.78	0.84	0.81	0.81	0.86	±0.05	NS
Spleen (g)	0.069	0.076	0.070	0.069	0.064	±0.015	NS

a, and b Means denoted within the same raw with different superscripts are significantly different at P<0.05.

NS: Not significant

Table (3): Some blood hematological traits of Inshas local chickens as affected by in ovo injection with different levels of L-arginine.

Parameters	Treatment groups					Sig. test
	G1	G2	G3	G4	G5	
RBCs (10 ⁶ /mm ³)	1.98±0.19 ^c	2.60±0.17 ^b	3.33±0.18 ^a	3.60±0.03 ^a	3.50±0.06 ^a	*
Hemo (g/dL)	9.02±0.24 ^b	9.53±0.18 ^b	11.05±0.21 ^a	11.18±0.28 ^a	11.25±0.72 ^a	*
PCV (%)	30.72±0.33 ^c	33.01±0.44 ^b	33.87±0.58 ^{ab}	34.53±0.30 ^a	34.38±0.22 ^a	*
WBCs (10 ³ /mm ³)	21.63±0.38 ^b	22.32±0.40 ^b	23.43±0.41 ^a	24.36±0.23 ^a	24.42±0.07 ^a	*
Heterophils (%)	21.04±0.18 ^b	21.32±0.40 ^b	22.45±0.74 ^{ab}	23.74±0.38 ^a	23.88±0.63 ^a	*
Lymphocytes (%)	71.33±0.88	69.67±3.71	69.67±1.20	70.83±3.24	69.67±0.88	NS
H/L ratio	0.295±0.006 ^c	0.307±0.011 ^b	0.322±0.011 ^{abc}	0.336±0.012 ^{ab}	0.343±0.009 ^a	*

a, b, c and d: Means denoted within the same raw with different superscripts are significantly different at P<0.05.

NS: Not significant

Table (4): Blood biochemical (With in normal range) of Inshas local chickens as affected by in ovo injection with different levels of L-arginine.

Parameters	Treatment groups					Sig. test
	G1	G2	G3	G4	G5	
Total proteins (g/dl)	2.61±0.09 ^b	2.72±0.06 ^b	3.09±0.28 ^b	3.66±0.15 ^a	3.82±0.05 ^a	*
Albumin (g/dl)	1.43±0.12 ^b	1.45±0.12 ^b	1.63±0.04 ^{ab}	1.73±0.03 ^a	1.79±0.06 ^a	*
Globulin (g/dl)	1.18±0.2 ^b	1.27±0.14 ^b	1.46±0.29 ^{ab}	1.93±0.163 ^a	2.03±0.1 ^a	*
AL/GL ratio	1.32±0.32	1.19± 0.2	1.19±0.21	0.91±0.09	0.89±0.07	NS
Glucose (mg/dl)	180.93±5.46 ^b	186.27±5.6 ^b	195.13±1.97 ^b	210.33±5.83 ^a	215.2±0.64 ^a	*
Total lipids (mg/dl)	456.27±0.81 ^a	442.1±0.52 ^a	345.77±18.89 ^b	301.93±5.21 ^c	292.5±6.03 ^c	*
Total cholesterol (mg/dl)	192.5±0.47 ^a	189.57±0.45 ^a	160.57±1.31 ^b	153.77±0.94 ^c	152.17±1.21 ^c	*
Triglyceride	195.2±0.55 ^a	193.37±1.46 ^a	173.3±0.89 ^b	152.57±0.93 ^c	149.43±1.30 ^c	*

a, b, and c: Means denoted within the same raw with different superscripts significantly different at P<0.05.

NS: Not significant

Table(5):Enzymatic activity in serum (With in normal range) of Inshas local chickens as affected by in ovo–injection with different levels of L–arginine.

Parameters	Treatment groups					Sig-test
	G1	G2	G3	G4	G5	
AST U/L	190.63±0.49	191.9±1.27	192.53±1.43	194.70±1.8	193.70±0.99	NS
ALT U/L	22.18±0.09	22.32±0.15	22.39±0.58	22.44±0.55	22.75±0.33	NS
AST/ALT	8.6±0.06	8.6±0.007	8.61±0.21	8.69±0.18	8.52±0.09	NS
ALP U/mL	621.5±3.21 ^c	627.6±4.09 ^c	675.7±2.06 ^b	686.9±1.56 ^a	688.97±1.77 ^a	*

a, b, and c: Means denoted within the same raw with different superscripts are significantly different at P<0.05.

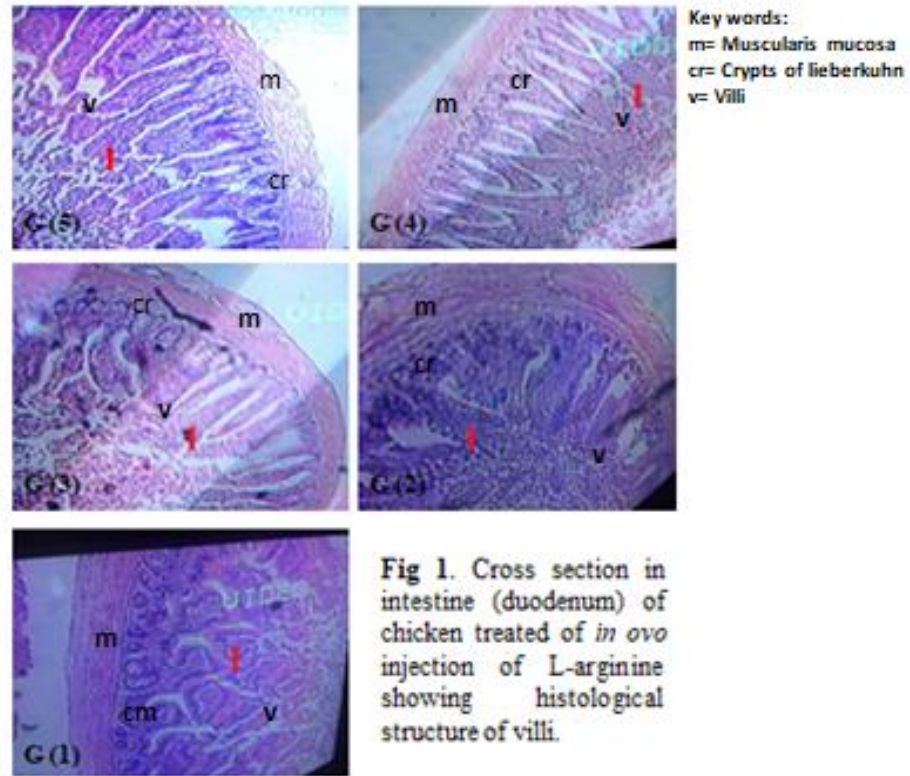
NS: Not significant

Table (6): Effects of by in ovo injection with L- arginine on histomorphometric measurements of small intestine in Inshas chicks.

Treatment groups	Parameters		
	Villus height, µm	Villus width, µm	Villus surface area, µm ²
G1	240.17±18.6 ^c	43.33±2.47 ^b	33209.5±3753.9 ^c
G2	252.67±9.95 ^{bc}	46.0±2.1 ^b	36352.4±1459.4 ^c
G3	286.67±6.54 ^b	55.83±2.39 ^a	50351.2±2605.9 ^b
G4	329.17±19.03 ^a	59.17±1.54 ^a	60958.3±3169.9 ^a
G5	342.17±2.95 ^a	61.67±1.67 ^a	66311.7±1874.2 ^a
Sig test	P<0.05	P<0.05	P<0.05

a, b, and c: Means denoted within the same column with different superscripts are significantly different at P<0.05.

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REFERENCES

- Abiola, S.S., Meshioye, O.O., Oyerinde, B.O. & Bamgbose, M.A., 2008.** Effect of egg size on hatchability of broiler chicks. *Arch. Zotech.* 57: 83-86.
- Alba-Roth, J., Muller, O.A. & Schophol, J., 1988.** Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *J. Clin. Endocrinol. Metabol.* 67: 1186-1189.
- Al-Daraji, H.J., Al-Mashadani, A.A., Al-Hayani, W.K., Al-Hassani, A.S. and Mirza, H.A., 2012.** Effect of in ovo injection with L-arginine on productive and physiological traits of Japanese quail. *South African Journal of Animal Science:* 42(2) 139-145.
- Al-Murrani, W. K., 1978.** Maternal effects on embryonic and postembryonic growth in poultry. *Br. Poult. Sci.* 19:277-281.
- Al-Murrani, W. K., 1982.** Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. *Br. Poult. Sci.*, 23:171-174.
- Bancroft, D., Stevens, A., and Turner, R., 1996.** Theory and practice of histology technique. 4th ed., Churchill Livingstone, Edinburgh, London, Melbourne.

- Bartell S. M., Batal A. B., 2007.** The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poult. Sci.* 86:1940-1947.
- Bhanja S., Mandal A., 2005.** Effect of in ovo injection of critical amino acids on pre- and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens. *Asian-Aust. J. Anim. Sci.*, 18: 524-531.
- Bhanja S.K., Mandal A.B., Agarwal S.K., Majumdar S., 2012.** Modulation of post hatch-growth and immunocompetence through in ovo injection of limiting amino acids in broiler chickens. *Indian J. Anim. Sci.*, 82: 993-8.
- Burke, W.H., Moore, J.A., Ogez, J.R. & 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. *Endocrin.* 120: 651-658.
- Campbell, B.I., LaBounty, P.M. & Roberts, M., 2004.** The ergogenic potential of arginine. *J. Int. Soc. Sport Nutr.* 1 (2), 35-38.
- Chen W., Wang R., Wan H.F., Xiong X.L., Peng P., Peng J., 2009.** Influence of in ovo injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. *Br. Poult. Sci.* 50: 436-442.
- Chevally, T, Rizzoli, R, Manen, D, Caverzasio, J, and Bonjour, J., 1998.** Arginine Increases Insulin-like Growth Factor-1 Production and collagen synthesis in Osteoblast-like Cells. *Bone* 23 (2):103-109.
- Coulombre, A.J, Coulombre J.L., 1958.** Intestinal Development. 1. Morphogenesis of the Villi and Musculature. *Journal of Embryology and Experimental Morphology* 6(3): 403-411.
- Cravener, T.L., Vasilatos-Younken, R. & Wellenreitter, R.H., 1989.** Effect of subcutaneous infusion of pituitary-derived chicken growth hormone on growth performance of broiler pullets. *Poult. Sci.*, 68:1133-1140.
- Darras, V.M., Berghman, L.R., Vanderpooten, S.M. & Kuhn, E.R., 1992.** Growth hormone actually decreases type III iodothyronine deiodinase in chicken liver. *FEBS Letters* 310, 5-8.
- Darras, V.M., Huybrchts, L.M., Berghman, L., Kuhn, E.R. & 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. *Gener. Compar. Endocrin.* 77: 212-220.
- Duncan, B.D. 1955.** Multiple range and multiple F test. *Biometrics* 11: 1-42.
- Ebrahimi, M., Janmohammadi, H., Daghigh, K. H., Moghaddam, G., Rajabi, Z., Abbas., R. S., Javanmard, A., 2017.** The effect of L-lysine in ovo feeding on body weight characteristics and small intestine morphology in a day-old Ross broiler chicks *Revue Méd. Vét.*, 168: 4-6, 116-124.
- Edwards, N.M., Heberle, N.D., Hynd P.I., 2016.** The Effect of In ovo Administration of L-Arginine on the Hatchability and Embryological Development of Broiler Chicks. *ASAP Animal Production*, Adelaide.

In ovo injection- hatchability-L-arginine, chicken

- Flakoll, P., Sharp, R., Baier, S., Levenhagen, D., Carr, C. & Nissen, S., 2004.** Effect of hydroxyl-methyl butyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism. *Nutr.*, 20: 444-451.
- Foye, O.T., Ferket, P.R. & Uni, Z., 2007.** The effects of in ovo feeding arginine, α -hydroxyl- β -methylbutyrate, and protein on jejunal digestive and absorptive activity in embryonic and neonatal turkey poults. *Poult. Sci.* 86: 2343-2349.
- Foye, O.T., Uni, Z. & Ferket, P.R., 2006.** Effect of in ovo feeding egg white protein, hydroxyl-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. *Poult. Sci.* 85: 1185-1192.
- Freeman, B. N., and Vince, M. A., 1974.** Development of Avian Embryo. Chapman and Hall, London, England. Rupe, C. O., and C. J. Farmer, 1955. Amino acid studies in the transformation of proteins of the hen's egg to tissue proteins during incubation. *J. Biol. Chem.* 213: 899-906.
- Gralak, M.A., Piastowska, A.W., Leontowicz, H., Leontowicz, M., Antczak, A., Kulasek, G., Szara, T. and Narojek, T., 2004.** Effect of dietary protein level and sources on bone mineralization and structure in rats. *Bio factors*, 22: 25-28.
- Harvey, S. & Etches, R.J., 1997.** Perspectives in Avian Endocrinology. J.W. Arrowsmith Ltd, Bristol, UK.
- Isidori, A., Lo Monaco, A, Cappa, M., 1981.** A Study of Growth hormone Release in Man after Oral Administration of Amino Acids. *Current Medical Research and* , 7(7): 475-481.
- Khajali, F., and Wideman, R.F., 2010.** Dietary arginine: metabolic, environmental, immunological, and physiological interrelationships. *World's Poult. Sci. J.*, 66: 751-766.
- Kim, Y.S., Kim, J.S., Cho, H.S., Rha, D.S., Kim, J.M., Park, J.D., Choi, B.S., Lim, R., Chang, H.K., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S. and Yu, I.J., 2008.** Twenty-eight-day oral toxicity, genotoxicity and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhalation Toxicology*, 20: 575-583.
- Libby, P. and Aikawa, M., 2002.** Vitamin C, collagen, and cracks in the plaque. *Circulation*, 105: 1396-1398.
- Narushin, V.G. and Romanov, M.N., 2002.** Egg physical characteristics and hatchability. *Journal of Wrl'd's Poultry Science*, 58: 297-302.
- Nouri Sanami, M., Ghaedi, B., Salary, J. and Hemati Matin , H.R., 2014.** In ovo injection of L-arginine on performance and bone mineralization in broiler chicken. *Res. Opin. Anim. Vet. Sci.*, 4(7): 394-397.
- Ohta, Y., Kidd, M.T. & Ishibashi, T., 2001.** Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. *Poult. Sci.* 80, 1430-1436.

- Ohta, Y., Tsushima, N., Koide, K., Kidd, M.T. & Ishibashi, T., 1999.** Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poult. Sci.* 78: 1493-1498.
- Petwket, 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 Sci.*, 33: 266–273.
- Pratt, D.S. and Kaplan, M.M., 2000.** Evaluation of abnormal liver-enzyme results in asymptomatic patients. *The New England Journal of Medicine*, 4: 1266-1271.
- Rupe, C. O., and Farmer, C. J., 1955.** Amino acid studies in the transformation of proteins of the hen's egg to tissue proteins during incubation. *J. Biol. Chem.* 213: 899–906.
- SAS, Institute 2001.** SAS User's Guide: Statistics version 6.12 ed. SAS Institute, Inc., Cary, NC., USA.
- Sohail, M., Hume, M., Byrd, J., Nisbet, D., Ijaz, A., Sohail, A., Shabbir, M. & Rehman, H., 2012.** Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poult. Sci.*, 91: 2235-2240.
- Surai, P.F., Noble, R .C., Speake, B. K., 1999.** Relationship between vitamin E Content and susceptibility to lipid peroxidation in tissues of the newly Hatched chick. *Br. Poult. Sci.*, 40: 406-410.
- Tako, E., Ferket, P.R. & Uni, Z., 2004.** Effects of in ovo feeding of carbohydrates and α -hydroxy- β -methylbutyrate on the development of chicken intestine. *Poult. Sci.*, 83: 2023-2028.
- Tong, B.C. & Barbul, A., 2004.** Cellular and physiological effects of arginine. *Mini Rev. Med. Chem.* 4 (8): 823-832.
- Uni Z., Ferket, R.P., 2004.** Methods for early nutrition and their potential. *Worlds Poult. Sci. J.*, 60: 101-111.
- Uni Z., Ganot S., Sklan D., 1998.** Posthatch development of mucosal function in the broiler small intestine. *Poult. Sci.*, 77: 75-82.
- Uni, Z., and Ferket, P.R. 2003.** Enhancement of development of oviparous species by in ovo feeding. North Carolina State Univ. and Yissum Research Development Company Assignees. US Pat. No. 6: 592-878.
- Uni, Z., Ferket, P., Tako, E. and Kedar. O. 2005.** In ovo feeding improves energy status of late-term chicken embryos. *Poult. Sci.*, 84: 764-770.
- Uni, Z., Tako, E., Gal-Garber, O. & Sklan, D., 2003.** Morphological, molecular and functional changes in the chicken small intestine of the late-term embryo. *Poult. Sci.*, 82: 1747-1754.
- Vaezi, G., Teshfam M., Bahadoran S., Farazyani H., and Hosseini S., 2011.** Effects of different levels of lysine on small intestinal villous morphology in starter diet of broiler chickens. *Glob. Vet.*, 7: 523-526
- Vandenbergh, K., Goris, M., Van Hecke, P. and Van Leemputte, M.A., 1997.** Long-term creatine intake is beneficial to muscle performance and pharmacokinetic considerations. *Journal of Applied Physiology*, 83: 2055-2063.
- Wu, G. & Morris, S.M., 1998.** Arginine metabolism: nitric oxide and beyond. *Biochem. J.*, 336: 1-17.

الملخص العربي

تأثير حقن ل- أرجنيين داخل البيضة على الفقس وبعض الصفات الفسيولوجية لدجاج سلالة أنشاص المحلية .

عبير أحمد عشره ، شريف مغاوري شاميه ، رجاء السيد عبد الكريم

معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية اجريت هذه الدراسة لتقييم تأثير حقن ل- أرجنيين بمستويات مختلفة (صفر، 1، 2، 3%) داخل البيضة في اليوم السابع من التفريخ وتأثير ذلك على نسبة الفقس وبعض الصفات الفسيولوجية على دجاج سلالة أنشاص المحلية. تم أخذ 500 بيضة مخصبه لدجاج أنشاص وتقسيمها الى خمس مجاميع متساويه كل مجموعه 100 بيضة على النحو التالي :

- 1- مجموعه 1 بدون حقن البيض G1 (كنترول سلبي)
- 2- مجموعه 2 حقن البيض بمحلول ملحي فقط G2 (كنترول إيجابي)
- 3- مجموعه 3 حقن البيض 1% ل- أرجنيين G3
- 4- مجموعه 4 حقن البيض 2% ل- أرجنيين G4
- 5- مجموعه 5 حقن البيض 3% ل- أرجنيين G5

اوضحت النتائج:

حدوث زيادة معنويه في نسبة الفقس في كلا من المجاميع (3و4و5) المحقونه بالارجنيين مقارنة بمجموعتي الكنترول.

حقن البيضة بالارجنيين نتج عنه زياده معنويه في كلا من الهيموجلوبين وعدد خلايا الدم البيضاء والحمراء في المجاميع المعامله بالمقارنه بمجموعتي الكنترول.

بينما كانت هناك زياده معنويه في الهيماتوكريت % و خلايا الهيتروفيل في المجموعه 5و4 بالمقارنه بمجموعتي الكنترول ومجموعه 3 .

لم يكن هناك تأثير معنويا على نسبة الخلايا الليمفاويه نتيجة حقن ل- أرجنيين بين المجاميع المختلفه . لوحظ في الكتاكيت الناتجه من البيض المحقون ل-أرجنيين في كلا من المجموعتي (4و5) حدوث زياده معنويه في تركيز الجلوكوز و البروتينات في السيرم بالمقارنه بباقي المجاميع وانخفاض معنوي للكولسترول والدهون الكليه والجلسريدات الثلاثية في (مجموعتي 4و5) بالمقارنه بمجموعه 3 ومجموعتي الكنترول .

طول ومساحه سطح الخملات ارتفعت معنويا فكلا من مجموعه 5و4 بالمقارنه بمجموعتي الكنترول ومجموعه 3 بينما عرض الخملات ارتفع معنويا في مجاميع 5و4و3 بالمقارنه بمجموعتي الكنترول .

الخلاصة : خلصت نتائج هذه الدراسه ان حقن البيض المخصب بالمستويات المستخدمه من الحمض الأميني ل- أرجنيين عند عمر 7 ايام التفريخ يؤدي الي حدوث تحسن معنوي في نسبة الفقس وبعض اوزان الذبيحه ومعايير الدم وكذلك عرض وطول وسطح الخملا