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EFFECT OF *IN OVO* ARGININE INJECTION ON GASTROINTESTINAL AND HAEMATO-BIOCHEMICAL INDICATORS OF FUNAAB-ALPHA CHICKENS

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ABSTRACT

This study investigated the effects of *in ovo* administration of arginine on duodenal villi development, weight gain and haemato-biochemical indices of FUNAAB-Alpha chickens. A total of 528 hatching eggs of FUNAAB-Alpha chickens were used in the experiment. The hatching eggs were balanced for weight, fumigated and, thereafter, set in the incubator. On 14th day of incubation, 378 eggs were confirmed fertile and assigned into 3 treatments (0, 11 and 22 mg of arginine/egg) each consisting of 126 eggs, replicated 14 times (9 eggs per replicate). At 18th day of embryonic age, *in ovo* administration of arginine was carried out and at 21st day of incubation, resulting chicks were balanced for weight on treatments basis, assigned to replicates and they were assessed for post-hatch performance. Data on gastro-intestinal development and haemato-biochemical indices were subjected to Completely Randomized Design. In the results, influence of *in ovo* injection of arginine (11 and 22 mg per egg) on duodenal histology of FUNAAB-Alpha chickens (at 14 days of age) resulted in marked improvement in the duodenal villi, as against that observed in birds under the control (without *in ovo* injection of arginine). Also, *in ovo* administration of arginine did not pose any deleterious effects on the haemato-biochemical indices of FUNAAB-Alpha chickens (at 4 weeks of age), as the values recorded were within normal ranges for healthy chickens. In conclusion, for enhanced performance in terms of duodenal villi, gastrointestinal development and haemato-biochemical indices of FUNAAB-Alpha chickens the *in ovo* injection of arginine (up to 22 mg/egg) is suitable without negative implications on the health status of the birds at 4 weeks of age.

Key words: *in ovo* injection; performance; health status; duodenal villi; gastro-intestinal tract; chickens

INTRODUCTION

Poultry production in Nigeria is confronted with diverse challenges including poor feed utilization, housing and health management among others. These challenges limit production and have a direct effect on availability of poultry products to the Nigerian teeming population. Therefore, introduction of innovative approaches to surmount these challenges and bring about improvement in poultry production is relevant. *In ovo* feeding, developed by Uni and Ferket (2004) is a technique whereby nutrients are administered to the developing embryo through the amniotic sac at later stage of embryonic development to provide a continuous supply of nutrients during perinatal period, thereby facilitating enteric development and metabolism. The authors patented *in ovo* feeding

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15 years ago but it has not been widely adopted by the poultry industry, particularly in Nigeria, for improving poultry production.

In practical commercial hatchery condition, eggs within a hatching crate will hatch over 24 – 36 hours (hatch window) during which birds that piped and hatch early are without feed. The early hatchings are disadvantage nutritionally due to prolonged fasting period. During this time, chicks loose in weight at the rate of 4 g per 24 hours arising from moisture loss as well as yolk and pectoral muscle utilization (Noy and Uni, 2010) This has profound negative effects on the bird's performance increasing their susceptibility to pathogens, weight loss (Noy and Sklan, 1999) and restricting the development of critical tissues (Moore *et al.*, 2005).

Amino acids are components of tissue proteins and provision of the different essential amino acids is a pre-requisite for maintaining optimal rates of protein synthesis. Arginine assists in the biosynthesis of biochemical molecules (protein, nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine and dimethylargininase) and hence, it is important in the regulation of several biological and physiological functions in poultry (Khajali and Wideman, 2010; Muhammad *et al.*, 2019). However, it has been reported that chickens cannot synthesize arginine due to lack of carbamoyl phosphate synthetase I in mitochondria (Lewis, 1996) and as a result, birds depend on dietary arginine to meet their needs for protein synthesis and other metabolic functions (Tamir and Ratner, 1963). D'Amato and Humphrey (2010) observed that inclusion of arginine in broiler diets activated the immune system and improved its efficiency in view of the benefits of arginine on birds' metabolic processes and physiological functions. This study, the effects of *in ovo* injection of arginine on duodenal villi development, weight gain and haemato-biochemical indices of FUNAAB-Alpha chickens were assessed.

MATERIAL AND METHODS

Experimental locations: the experiment was carried at the Hatchery and Poultry Unit of the Teaching and Research Farm, Federal University of Agriculture, Abeokuta (FUNAAB) Ogun State, located within Latitude 7° 15' 59.66" N, Longitude 3° 26' 13.64" E (Google Earth, 2019). The laboratory studies were carried out at the Anatomy and Physiology Laboratory, Department of Veterinary Physiology and Pharmacology, FUNAAB.

Source of hatching eggs: five hundred and twenty-eight (528) hatching eggs were sourced from breeder hen of FUNAAB Alpha chickens (age of 38 week) at the College of Animal Science and Livestock Production (COLANIM), PEARL FUNAAB Indigenous Chicken Breeder Farm, Federal University of Agriculture Abeokuta.

Hatchery preparation: incubator, its components (setting trays and hatching crates) and the hatchery surroundings were cleaned and disinfected. Thereafter, they were fumigated with formaldehyde and potassium permanganate ($KMnO_4$) in the ratio 2:1.

Setting and hatchery management of hatching eggs: the hatching eggs were sorted and balanced for weight. Subsequently, eggs were fumigated in an enclosed chamber before setting. The set eggs were managed with provision of temperature (37.50 °C), relative humidity (60 %) and turned automatically for 18 days in the setter prior to hatching at 21st day of incubation.

Assignment of hatching eggs to in ovo arginine treatments: Candling at 14th day of incubation revealed that a total of 378 eggs contain living embryos (fertile). The fertile eggs were assigned to three treatments with each having 126 eggs, replicated 14 times (9 eggs per replicate). *In ovo* arginine treatments consist of control (without *in ovo* injection); *in ovo* injections of 11 mg and 22 mg of arginine were dissolved in 0.5 ml of deionized water and administered *in ovo* per egg. The experiment was performed in a completely randomized design.

Procedure for in ovo arginine injection: at 18th day of incubation, eggs were injected with arginine into amnion using 24-gauge hypodermic needle (25 mm long) according to the procedure of Bhanja *et al.* (2004). Prior to injection, the injection points on the eggs (broad end) were sterilized with 30 % ethanol. The *in ovo* injection of each treatment was completed within 15 minutes of taking out the eggs from the incubator. After *in ovo* feeding, the injection sites on the eggs were sealed with candle wax and the eggs were transferred to hatching compartment where they were managed till hatching at 21st day of incubation.

Post-hatch management of birds: After hatching, 330 chicks resulting from *in ovo* injections of arginine were assigned to 3 *in ovo* treatments (control, 11 and 22 mg of arginine *in ovo* injections) with each replicated 5 times (22 birds per replicate).

The birds were brooded for three weeks, and managed intensively (deep litter housing) with the provision of feed (Table 1) and water *ad-libitum*. The experiment lasted for a period of 4 weeks.

Table 1. Percentage Composition of Diets

Ingredients	Diet
Maize	58.60
Soybean meal	36.10
Fats and oil (vegetable oil)	1.65
Limestone	1.00
Bone meal	1.75
Salt	0.35
Lysine	0.10
Methionine	0.20
*Premix	0.25
Total	100.00
Calculated Composition	
Metabolizable energy (kcal.kg^{-1})	2881.50
Crude Protein (%)	21.89
Crude Fibre (%)	3.93
Ether Extract (%)	3.61
Ash (%)	2.93

*Premix contains Vit. A, 10,000 000 IU; D_3, 2 000 000 IU; E, 12 500 IU; K, 1.30 g; B_1, 1.30 g; B_2, 4 g; D calcium pantothenate, 1.3 g; B_6, 1.3 g; B_{12}, 0.01 g; nicotinic acid, 15 g; Folic acid, 0.05 g; biotin, 0.02 g; Cu, 0.05 g; Co, 0.20; Fe, 25 g; I, 0.06 g; Mn, 48 g; Se, 0.10 g; Zn, 45 g; Choline chloride, 200 g; BHT, 50 g.

Data Collection

Assessing duodenal histology of the gastro-intestinal tract: this was evaluated by histological examination of duodenal villi. Two centimeters (2 cm) long portion of the duodenum was cut and placed into sample bottle containing 10 % formaline saline (100 ml formalin, 900 ml distilled water, 0.4 g sodium dihydrogen phosphate, 0.65 g disodium hydrogen phosphate) after washing the contents with normal saline. The duodenal cut samples were prepared as slides for light microscopy, as described by Shamoto and Yamauchi (2000).

Evaluation of gastro-intestinal tract development: At 14th day of age, two birds of average weight from each replicate were selected and slaughtered by cervical dislocation for gut developmental studies. Gastrointestinal tract morphometry was evaluated by recording the weights (using a sensitive scale) of proventriculus, empty gizzard, as well as the weight and length of the intestine expressed in cm/100 g live weight (Sogunle *et al.*, 2018).

Determination of haemato-biochemical indices: At 4th week of age, two (2) birds of average weight of each replicate were selected for blood collection; 3 ml of blood sample were collected from each bird using wing web vein puncture method. Blood samples collected were divided into two portions (EDTA and plain bottles) for the determination of haematological (Haemoglobin, Red blood cell, Packed cell volume, White blood cell and its differentials) and serum biochemical (total protein, albumin, globulin, triglyceride, cholesterol, AST, ALT and ALP) indices using standard procedure (Jain, 1986).

Data Analysis: Data collected on gastrointestinal tract development and haemato-biochemical indices were subjected to one-way analysis of variance, and significantly ($P < 0.05$) differed means among variables were separated using Tukey-test in Minitab® version 17.1.0.

RESULTS AND DISCUSSION

Figure 1 represents the duodenal villi of FUNAAB-Alpha chickens under the control treatment. In Plate 1, there are moderate numbers of short slender villi (red arrow). The cryptal glands (black arrow) appear normal.

In Figure 2, (duodenal villi of birds resulting from *in ovo* injection of 11 mg of arginine), there are numerous tall slender villi (black arrow) with folded tips and cryptal glands (green arrow) also appear regular.

Figure 3 shows the duodenal villi of birds from fertile eggs injected 22 mg of arginine. Moderate numbers of short plump villi (black arrow) are observed. The cryptal glands (green arrow) are normal and there are mild increases of mononuclear cells in the pericryptal regions.

The influence of *in ovo* injection of arginine (11 and 2 mg per egg) on duodenal histology of FUNAAB-Alpha chickens (at 14 days of age) revealed

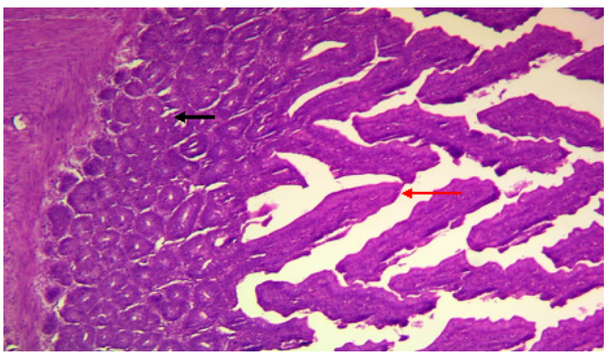

Figure 1. Duodenal villi of FUNAAB-Alpha chickens under control (without *in ovo* injection of arginine) at 14 days age

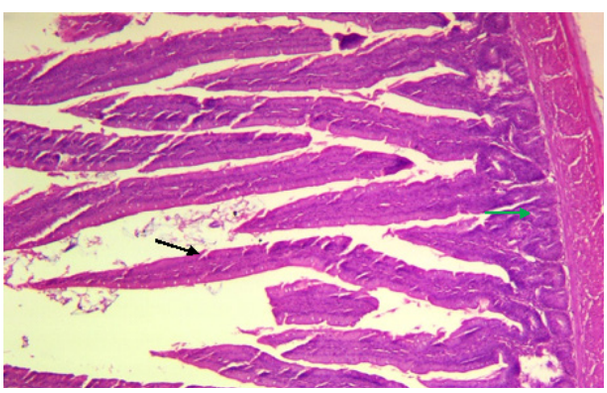

Figure 2. Duodenal villi of FUNAAB-Alpha chickens (at 14 days of age) resulting from fertile eggs injected 11 mg of arginine

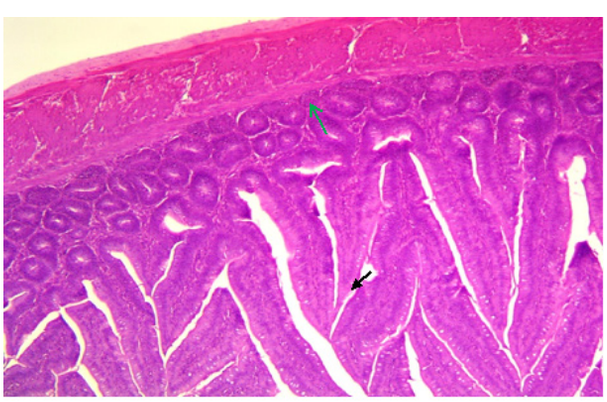

Figure 3. Duodenal villi histology of FUNAAB-Alpha chickens (at 14 days of age) resulting from fertile eggs injected 22 mg of arginine

marked improvement in the duodenal villi with moderate or numerous tall villi and cryptal glands that are normal as against birds under the control.

Variations noted in the duodenal villi of birds depict the positive influence of arginine on promoting growth of the intestinal villi in the birds. The growth enhancing attribute of arginine is attributable to its function as a primary component of body protein owning to its ability to stimulate growth hormone and insulin like growth factor secretion into the blood stream (Silva *et al.* 2012), which promotes cell division, protein synthesis and tissue growth (Pegg and McCann 1982). Sogunle *et al.* (2019) found that *in ovo* injection of amino acids (arginine + methionine) resulted in improvement of the duodeneal villi histology (several tall villi and crypts) of broiler chicks at 7 days of age. Awachat *et al.* (2017) reported increased villi heights in the duodenum and ileum of birds resulting from *in ovo* injection of arginine (22 mg) and arginine + threonine (22 mg + 30 mg). *In ovo* feeding with 2 % arginine significantly improved body weight gain and feed intake of broiler chicks (Al-Asadi, 2013). Foye *et al.* (2007) confirmed that *in ovo* feeding with a solution containing 0.7 % arginine increased the digestive enzyme activity produced by pancreas leading to improved growth performance in turkey poultry. Al-Daraji *et al.* (2012) observed that quails, obtained from eggs injected with arginine (2 and 3 %), had higher body weight at 7 day age and 6 weeks of age, than those from control eggs. *In ovo* injection of IGF-I significantly improved small intestine sucrose concentrations in all sections of small intestine in broiler chickens at 21 and 42 days of age (Moosavinasab *et al.*, 2015).

Table 2 represent the effects of *in ovo* injection of arginine on the development of the gastrointestinal tract of FUNAAB-Alpha chickens. No significant ($P > 0.05$) variations were observed in all organs and gut morphological parameters measured. It was, however, observed that values for liver (3.20 %), gizzard (6.87 %), proventriculus (0.94 %), duodenum percent (1.91 %), duodenum length (10.47 cm/100 g live weight), jejunum percent (2.27 %), ileum percent (2.26 %), ileum length (19.80 cm/100 g live weight) and colon length (3.31 cm/100 g live weight) were numerically higher in chicks derived from an *in ovo* injection of 11 mg of arginine.

Similar finding was observed by Dror *et al.* (1997) that the highest relative size of digestive organs in chicks occurs at 3 – 7 days of age. Sogule *et al.* (2018)

Table 2. Effect of *in ovo* injection of varying levels of arginine on gastrointestinal tract development of FUNAAB-Alpha chickens at 14 days of age

Parameter	Control	11 mg Arginine/egg	22 mg Arginine/egg	SEM	*P*-value
Live weight (LW) (g/bird)	141.80	138.80	150.30	12.70	0.805
Organs[1]					
Liver (%)	3.06	3.20	3.02	0.21	0.819
Empty gizzard (%)	6.66	6.87	6.09	0.52	0.570
Heart (%)	0.88	0.89	0.99	0.09	0.619
Proventriculus (%)	0.82	0.94	0.87	0.87	0.367
Gastrointestinal tract					
Duodenum (%)	1.64	1.91	1.71	0.17	0.514
Duodenum length (cm/100g LW)	9.65	10.47	9.62	0.88	0.748
Jejenum (%)	2.19	2.27	1.88	0.21	0.421
Jejenum length (cm/100g LW)	19.97	18.58	18.06	1.19	0.525
Ileum (%)	1.48	2.26	1.91	0.28	0.194
Ileum length (cm/100g LW)	17.88	19.80	18.04	1.56	0.641
Caecum (%)	1.27	0.70	0.81	0.23	0.222
Colon (%)	0.43	0.42	0.45	0.10	0.967
Colon length (cm/100g LW)	2.57	3.31	3.24	0.30	0.210

[1]Values expressed as percentage of live weight.

and Odutayo *et al.* (2020) reported that *in ovo* injection of arginine in organic salts of minerals (Zn, Se and Cu) resulted in insignificant differences in organs and gut morphological parameters measured in broilers chicks at 7 and 35 days of age, respectively. Bhanja *et al.* (2012) observed that *in ovo* injections of lysine, methionine, threonine, arginine and glycine, each given at 25 mg, led to insignificant variations in weight of digestive organs and intestine in 1-day old chicks. However, Nayak *et al.* (2015) found significant variation only at 4 day of post-hatch in the duodenum and ileum lengths of broiler chickens derived from an *in ovo* injection of arginine and or tryptophan.

The influence of *in ovo* arginine injection on haemato-biochemical indices of FUNAAB-Alpha chicken is shown in Table 3. *In ovo* injection of arginine did not significantly ($P > 0.05$) affect any of the measured haemato-biochemical indices. This is in contrary to the report of Al-Daraji *et al.* (2012), who observed significant differences in serum metabolites (glucose, protein, cholesterol, triglyceride, calcium and phosphorus) of Japanese quails resulting from an *in ovo* injection of varying levels (0, 1, 2 and 3 %) of arginine. Sahr *et al.* (2020) also documented that *in ovo* injection of inorganic salts (Zinc, Copper and Manganese) leads to significant variation only in basophil (at 7th days of age) as well as white blood cells (at 42nd days of age) in broiler chickens. However, range of values obtained for these parameters were similar with those published by previous authors. The packed cell volume (PCV) value, ranged from 31.25 to 36.75 %, is within the PCV range (24.9 to 45.2 %) for healthy chickens, reported by Mitruka and Rawnsley (1977), Nworgu *et al.* (2007) and Riddell (2011).

Haemoglobin (Hb) values, observed for the FUNAAB Alpha chickens, ranged from 10.42 to 12.28 g.dl^{-1}, which is consistent with 7.4 – 13.1 g.dl^{-1} Hb, reported by Mitruka and Rawnsley (1977). Haemoglobin values of 11.50 to 13.50 g.dl^{-1} were reported for local grower Nigerian chicken (Afolabi *et al.*, 2011). Range of values (2.63 to 3.08 × 10^6/mm^3) for red blood cell (RBC) is similar to 1.58 to 4.1 x 10^6/mm^3, documented by Mitruka and Rawnsley (1977). Afolabi *et al.* (2011) observed the RBC to range in 2.82 to 3.37 x 10^6/mm^3 for local grower chicken in Nigeria. Also, values obtained for the white blood

Table 3. Effect of *in ovo* injection of varying levels of arginine on haemato-biochemical parameters of FUNAAB-Alpha chickens at 4th weeks of age

	In ovo Arginine injection				
Parameter	Control	11 mg Arginine/egg	22 mg Arginine/egg	SEM	*P*-value
PCV (%)	36.75	35.75	31.25	1.48	0.065
Hb (g/dl)	12.28	11.78	10.42	0.50	0.072
RBC ($\times 10^6$ / mm^3)	3.08	3.03	2.63	0.12	0.046
WBC ($\times 10^6$ / mm^3)	13.25	13.58	12.80	1.50	0.935
Heterophils (%)	39.00	34.75	33.00	2.04	0.163
Lymphocytes (%)	57.50	64.50	66.00	2.16	0.051
Eosinophils (%)	1.00	0.25	0.75	0.40	0.443
Basophils (%)	1.00	0.25	0.10	0.14	0.060
Monocytes (%)	1.50	0.26	0.00	0.42	0.075
Total protein ($g.dl^{-1}$)	2.88	3.13	2.96	0.18	0.740
Albumin ($g.dl^{-1}$)	1.88	1.78	1.58	0.11	0.226
Globulin ($g.dl^{-1}$)	1.00	1.35	1.38	0.21	0.417
Cholesterol ($g.dl^{-1}$)	140.55	130.05	121.87	8.40	0.339
Triglyceride ($g.dl^{-1}$)	125.80	107.58	131.48	6.01	0.054
ALP (U/l)	32.25	30.75	26.75	2.19	0.246
AST (U/l)	41.25	39.75	45.50	1.76	0.115
ALT (U/l)	22.50	21.25	21.00	4.05	0.961

PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cells; WBC: White blood cells.
ALP: Alkaline phosphatase; AST: Aspartate amino transferase; ALT: Alanine amino transferase.

cells, its differential counts and serum metabolites were comparable to those reported by other authors (Mitruka and Rawnsley, 1977; Ross *et al.*, 1978; Ikhimioya *et al.*, 2000; Riddell, 2011; Alabi *et al.*, 2015) depicting healthy conditions for domestic chickens. Basing on the values of the haemoto-biochemical indices, reported for healthy chickens, it is obvious that *in ovo* administration of arginine (at 11 and 22 mg) did not have any deleterious influence on the health characteristics of the birds.

CONCLUSION

In ovo injection of arginine, given at 11 or 22 mg/egg, is appropriate for improved performance, with respect to duodenal villi development and haemato-biochemical indices of FUNAAB-Alpha chickens (up to 4th week of age), without adverse implications on the health status of the birds.

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