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Effects of *in ovo* injection of amino acids on hatching performance, cell-mediated immunity and blood profile of FUNAAB Alpha broiler chickens

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Abstract

In the study, the effects of in ovo injection of amino acids (L-methionine, L-lysine, and L-arginine) and their combinations on hatching traits, post-hatch performance, cell-mediated immune response and blood profile of FUNAAB Alpha broiler chicken were evaluated. The study was carried out in two trials. In the first trial, a total of 360 hatching eggs of indigenous chicken were fumigated, weighed and placed in the incubator. On day 14 of incubation, candling was done and eggs with living embryos were distributed into four treatments; Control (un-injected eggs), L-methionine injected eggs, L-arginine injected eggs and L-lysine injected eggs. Each egg was injected 0.5 mL solution of the amino acid on day18. Hatched chicks were distributed into three replicates containing 30 chicks each. In trial 2, another 360 hatching eggs were used in the treatments which were; Control (eggs without amino acid injection), L-arginine injected eggs, combination of L-arginine and L-methionine injected eggs and combination of L-arginine and L-lysine injected eggs. On day 21 post-hatch, cell-mediated immune response, haematological and serum biochemical parameters were determined. Data obtained from the two trials were subjected to Completely Randomized Design. Results revealed the highest hatchability of 70.27% in the control treatment (un-injected), followed by 51.35% in arginine-injected eggs with the lowest (2.70%) in methionine-injected eggs. Arginine was found to enhance hatchability while L-methionine injected in ovo decreased hatchability. In the second trial, the results showed highest hatchability (89.58%) in eggs under the control, followed by 39.29%, 30.80% and 21.43% in eggs injected with the combination of arginine and lysine, arginine injected eggs, and those with arginine and methionine combination, respectively. Significant ($p<0.05$) differences in growth performance were only observed in the feed conversion ratio and percentage survivability. Better feed conversion ratio of 2.18 and 2.29 were recorded in birds injected with arginine and the control as against those subjected to combined arginine and methionine (2.96) as well as combined arginine and lysine (3.12). Survivability of 100% was recorded in chickens from arginine-injected eggs in ovo injection and chickens from eggs injected with the combination of arginine and methionine. The study concluded that in ovo injection of arginine either singly or in combination with lysine or methionine positively influenced hatchability, chick weight and growth performance without any deleterious effect

on the blood profile of the post-hatch chicks.

Keywords: L-arginine, L-methionine, L-lysine, Cell-mediated Immunity, Blood profile, Duodenal histology, Indigenous Chicken

Introduction

There is a growing concern on how the genetic resources of indigenous chickens can be improved for better performance. The indigenous chickens are commonly found in developing countries including Nigeria and they play a vital role in many rural households. However, productivity of these chickens is low, thus they produce few eggs which have low hatchability (Safalaoh, 2001). Although, they are mostly raised on free range or in some cases on semi-intensive system of management, their production does not commensurate the investment. Poor nutrition plays an important role in all these problems and thus, the production potential of these breeds cannot be realized without supplying them with required nutrients. This contributes to low income and poor nutrition for rural poor resource farmers. FUNAAB Alpha broiler chicken was developed as an improvement over the indigenous chickens by a foremost scientist in the field of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria.

Nutrition is a crucial growth performance component that can be manipulated in order to improve poultry production. Several strategies on nutrition have been looked into in order to achieve economic and better performance in poultry production. One of such nutritional strategies is *in ovo* feeding of exogenous material which was first carried out in 1980s, with vaccination of poultry against Marek's disease (Sharma and Burmester, 1982). *In ovo* feeding is a technology that gives hatchlings a nutritional benefit as a means of introducing nutrients into developing embryo (Uni and Ferket, 2003). Among several importance of *in ovo* feeding from previous reports include; better efficiency of feed utilization (Bhanja *et al.,* 2004); reduce post-hatch mortality and morbidity; improved immune response (Gore and Quereshi, 1997); enhanced early growth by improving intestinal function and development by enhancing absorption by the villi (Tako *et al.,* 2004; Noy and Uni, 2010); increased skeletal growth and breast muscle yield (Hajihosaini and Mottaghitalab, 2004), marketing body weight (Selim *et al.,* 2012) and improved hatchability (Sogunle *et al*., 2018). Different nutrients are used for *in ovo* feeding including amino acids to promote growth, immune and gut functions. Methionine is involved in the metabolic processes that improve health, growth, development and reproduction in animals. Being the first limiting amino acid in poultry diets, methionine affects poultry production (Jankowski *et al.,* 2014). Dietary methionine supplementation to broiler diets improved feed efficiency and body weight gain (Maatman *et al.,* 1993); increased carcass yield (Schuttle *et al.,* 1997); and live weight gain (Safaei *et al.,* 2012). Lysine as well contribute to the synthesis of body proteins and peptides, which are indispensable organic compounds participating in all biochemical reactions and physiological activities, including structural support of living cells and tissues. Khajali and Wideman (2010) opined that arginine serves as substrate for the biosynthesis of several molecules, including protein, nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine and dimethylargininese thereby playing a vital biological and physiological function in poultry. Also the inclusion of arginine in poultry diets is essential in order to keep the

birds away from the harmful influences of free radicals produced during normal metabolism (Atakisi *et al.,* 2009). These nutrients are supplemented to the developing embryo through the amniotic sac at the 18th day of incubation, to provide a continuous supply of critical nutrients during the first few days after hatching, and thereafter help in enteric development and metabolism process. Supplementing the amnion fluid with nutrients by the process of *in ovo* injection of amino acids (proteins) will go in long way to correct inadequacies in poultry diets, positively affect hatching eggs nutrients status and enhance the overall performance of the resulting chicks. This study therefore determined the effects of *in ovo* feeding of amino acids on hatching, post-hatching performance, cell-mediated immune response, and blood profile of indigenous chickens.

Materials and methods

Experimental location

The study was composed of two trials as described; *In ovo* injection of DL-methionine, L-lysine, L-arginine and their combination in hatching eggs of improved indigenous chicken and *In ovo* injection of L-arginine, combined L-arginine and DL-methionine; combined L- arginine and L-lysine.

The hatchery study was carried out using the Incubator of the College of Animal Science and Livestock Production. The field experiment was undertaken at the Poultry unit of the Teaching and Research Farm of the University while the laboratory study was carried out at the Animal Products and Processing Laboratory of the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Source of fertile eggs of indigenous chicken

A total of 360 hatching eggs of indigenous chickens sourced from the Teaching and Research Farm, Federal University of Agriculture, Abeokuta was used at each trial.

Management of the fertile eggs at the hatchery

The 360 hatching eggs were handled carefully and store at room temperature, they were weighed individually before being transferred to the setting compartment. These hatching eggs were divided into four treatments (90 eggs per treatment) groups at each trial. Each treatment is replicate three times. Description of the treatment group for the separate trial is represented as follows:

In ovo injection for trial 1 was composed of the following treatment groups**:** Control (un-injected), L-arginine (25 mg/egg), L-methionine (10 mg/egg) and L-lysine (22 mg/egg). In trial 2, the following treatment groups were used: Control (un-injected), L-arginine (25 mg), Combined L-arginine and L-methionine (25 mg + 10 mg/egg) and Combined L-arginine and L-lysine (25 mg + 22 mg/egg). To avoid contamination, the eggs were fumigated with potassium tetra-oxo-manganate VII ($KMNO_4$) and formaldehyde in the ratio 1:2 in an enclosed chamber for just 20 minutes. Thereafter, the eggs were placed inside an incubator and managed (with the appropriate temperature, relative humidity and turned automatically on hourly basis) for 18 days.

Preparation of amino acids solution for in ovo injection

A total of 10 mg of methionine, 22 mg lysine and 25 mg of arginine were dissolved in 0.5 mL of deionised water as described by Bhanja *et al*. (2012).

Procedure for in ovo amino acids injection

At 14 days of incubation, the 360 eggs were checked for fertility by candling and the dead embryos were equally removed from the incubator. The eggs with living embryo were redistributed into four treatments groups as described above. At day 18 of incubation, the broad end of each egg was

cleaned with mild disinfectant (30% ethanol) and a pinhole was made on the broad end of the egg. Thereafter, 0.5 mL of the amino acid solutions were injected into the egg with a 24-gauge hypodermic needle (11 mm long) (Bhanja *et al*., 2004). The holes were sealed up with sterile paraffin and the eggs were returned into the incubator. The exercise was completed within 30 minutes of taking out of eggs from the incubator. The injected eggs including the eggs on control treatment were then transferred to hatching compartment. Proper monitoring of the incubator conditions was done.

Post-hatched chicks

The chicks that were hatched on day 21 of incubation, were weighed to evaluate the effect of *in ovo* injection of the amino acids. They were distributed into the four treatments, replicated three times with equal number of chicks per replicate. They were managed intensively in deep litter with the provision of feed (Table 1) and water *ad libitum*.

Data collection

Data were collected on the hatching traits which included egg weight, percentage hatchability, chick weight, chick to egg ratio and embryonic mortality. The feed intake, weight gain and mortality were recorded daily while the feed conversion ratio was calculated.

Histology of the duodenum

A segment of the duodenum from the proximal and distal parts of the ampulla was selected from two birds slaughtered by cervical dislocation at age 7 days of the study in trial 2. The thin parts were collected from five regions: proximal, proxo-middle, middle, mid-distal and distal. The samples were fixed in 4% paraformaldehyde in PBS for 30 to 40 hours at room temperature for routine histological techniques. After fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax as blocks as described by Althnaian *et al.* (2013)

Table 1: Composition (%) of the experimental diets

Ingredients	1-5 Weeks	6-10 Weeks
Maize	58.20	62.50
Soybean meal	36.10	31.50
Fats and oil	1.65	2.20
Limestone	1.00	1.10
Bone meal	1.75	1.75
Salt (NaCl)	0.35	1.35
Lysine	0.10	0.12
Methionine	0.20	0.20
*Vitamins and Minerals Premix	0.25	0.28
Total	100.00	100.00
Calculated composition		
M E (Kcal/Kg)	2991.50	3047.75
Crude Protein (%)	21.89	20.07
Crude Fibre (%)	3.92	3.70
Ether extract (%)	5.37	5.92
Ash (%)	2.92	2.68

**Vit. A 12,500,000 iu; Vit. D $_3$ 2, 5000,000 iu; Vit E 40,000mg; Vit. K 32,00 0mg; Vit. B $_1$ 3,000 mg; Vit. B $_2$ 5,500 mg; Niacin 55,000 mg; Calcium Pentothenate 11, 5000 mg; Vit. B $_6$ 5,000 mg; Vit. B $_{12}$ 25 mg; Choline Chloride 500,000 mg; Folic acid 1,000 mg; Biotin 80 mg; Manganese 120,000 mg; Iron 100,000 mg; Zinc 80,000 mg; Copper 8,5 000 mg; Iodine 1,500 mg; Cobalt 300 mg; Selenium 120 mg; Anti-oxidant 120,000 mg.*

Cell-mediated immune response

Cell-mediated immunity was carried out at day 21 post-hatch. The reagent used was: Phosphate buffer saline (PBS); Sodium chloride, 8.0 g: Potassium chloride, 0.20 g: Potassium dihydrogen phosphate, 0.20 g; Disodium hydrogen phosphate. 1.44 g; distilled water, 1 litre; pH of 7.2. The method of Corrier and Deloach (1990) was used which shows the response of cell-mediated immune to phytohemagglutinin type P (PHA-P). At 21 days post-hatch, 0.1ml (concentration 1 mg/ml) of PHA-P was injected at both the third and fourth inter-digital space of the right foot of the sampled bird. While the left foot which serve as control was injected with 0.1 ml of phosphate buffer saline (PBS). The foot web index was calculated as the difference between the swelling in the right and left feet before and after 24 hours of injection and expressed as millimetre.

Cell-mediated immune response (CMIR) = (R2-R1) – (L2-L1)

R2 = Thickness of right foot web after 24 hours of injection

R1 = Thickness of the right foot web before injection.

L2 = Thickness of left foot web after 24 hours of injection

L1 = Thickness of the left foot web before injection

Determination of haematological parameters and serum biochemical indices

On day 21 of trial 2, 5 mL of blood were collected from the brachial vein of 3 selected birds per replicate into Ethylene Diamine Tetra acetic acid (EDTA) tubes. All samples were collected in the morning before feeding (between 07:00 am to 09:00 am). Blood samples collected were kept in cool containers and transported to the laboratory within 2 hours of blood withdrawal. Haematological parameters measured were analyzed according to the procedures described by Sood (2016). Packed Cell Volume (PCV) was determined using microhaematocrit capillaries. Haemoglobin concentration (Hb) was determined using cyanmethaemoglobin method which involves mixing 5 mL of Drabkin's solution (1000 mL of deionised water was mixed with 400 mg of Potassium ferricyanide, 280 mg of Potassium dihydrogen phosphate, 100 mg of Potassium cyanide and 1 mL of non-ionic detergent) with 20 µl of blood sample. The mixture was read in a photocolorimeter at 540 nm (green filter). Blood counts were determined using the improved Neubauer's chamber (area of 9 sq/mm and depth of 0.1 mm). Platelet count was determined using Rees-Ecker method using a diluting fluid that consist of 3.8 gm of Trisodium citrate, 0.2 ml of Neutral formaldehyde, 0.1 gm of Brilliant cresyl blue and 100 mL of Deionised water.

Serum biochemical parameters (total protein, albumin, globulin, creatinine, Alanine transaminase (ALT) and Aspartate transaminase (AST), uric acid, cholesterol, triglycerides) were analyzed using commercially available test kits by Randox laboratories, United Kingdom (Model BT294QY).

Experimental design

The experiments were laid out in a Completely Randomized Design.

Statistical analysis

Data were subjected to Analysis of Variance. Significantly ($P<0.05$) different means among variables were separated using Tukey as contained in Minitab (2013) version 17.1.0.

Results

Figure 1 shows the effect of *in ovo* injection of amino acids on hatching traits of indigenous chickens for trial 1. The highest hatchability of 70.27 % was obtained in hatching eggs of indigenous chicken in the control treatment followed by hatching egg subjected to arginine *in ovo* injection (51.35

%), while the lowest hatchability percentage of 2.70 was observed from hatching eggs of indigenous chicken under methionine *in ovo* injection. Highest chick weight (39.31g) was recorded in chicks hatched in the control treatment group while the lowest chick weight of 31 g was obtained from chicks injected with methionine. The numerically highest chick to egg ratio of 0.70 was recorded in hatching eggs injected with lysine while the lowest (0.54) chick to egg ratio was noted in hatching eggs of indigenous chickens under methionine injection. Figure 2 shows the effect of *in ovo* injection of arginine, its combination with methionine and lysine on hatching traits of indigenous chickens for trial 2. Hatchability was highest (89.58%) in eggs on the control treatment followed by 39.29% in eggs injected with the combination of arginine and lysine, and the lowest hatchability (28.57%) was recorded in eggs injected with the combination of arginine and methionine.

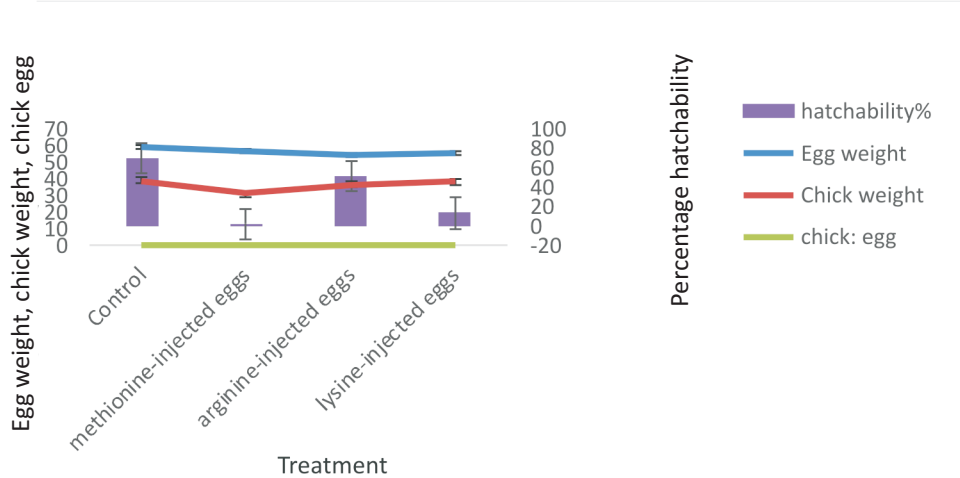

Fig. 1: Effect of in ovo injection of methionine, arginine and lysine on hatching traits of FUNAAB Alpha broiler chickens

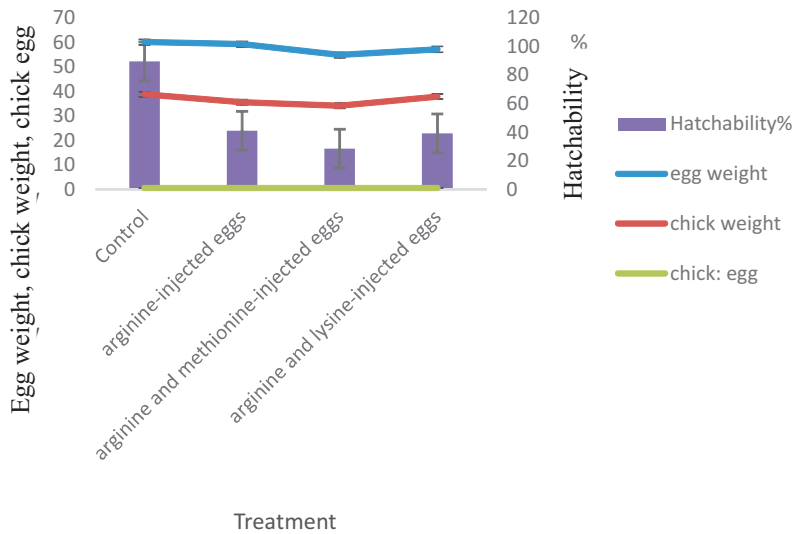

Fig. 2: Effect of in ovo injection of arginine, its combination with methionine and lysine on hatching traits of FUNAAB Alpha broiler chickens

Table 2 shows the effects of *in ovo* injection of amino acids on the growth performance of indigenous chickens. Significant ($p<0.05$) differences were observed in the feed conversion ratio and percentage survivability. Better feed conversion ratios of 2.18 and 2.29 were recorded in birds injected with arginine and the control as against the value obtained in birds subjected to *in ovo* injection of combined arginine and methionine and combined arginine and lysine amino acids. The best survivability was noted in indigenous chickens on *in ovo* injection of arginine and the combination of arginine and methionine amino acids. In Figure 3, the result showed that the highest cell-mediated immune response of 0.123 mm was recorded in chicken injected with combined arginine and lysine. This was followed by birds subjected to *in ovo* injection of arginine (0.116 mm) and the lowest cell-mediated immune response of 0.054 mm was obtained in birds on *in ovo* injection of the combination of arginine and methionine amino acids. Plates 1, 2, 3 and 4 show the effects of in ovo injection of amino acids on duodenal histology of improved indigenous chickens at 7 days of age. In Plate 1, moderate number of duodenal villi were observed with varying heights. Few foci of mild sloughing off of enterocytes are prominent. In Plate 2, there are few tall duodenal villi (red arrow) and the crypts are also high (blue arrow). Plate 3 reveals numerous tall duodenal villi (red arrows) of improved indigenous chickens. Furthermore, the plate shows that enterocytes appear intact without been affected. Moderate congestion of the blood vessels in the lamina propria (green arrow) and crypts are slightly high (blue arrow) and in Plate 4, moderate amounts of short duodenal villi (red arrows) and reduced crypts (blue arrow) are obtained. There is moderate expansion of lamina propria (green arrow). Table 3 shows the effect of *in ovo* injection of amino acids on blood parameters of indigenous chickens. There were no significant ($P>0.05$) differences in all parameters considered.

Table 2: Effect of *in ovo* administration of amino acids on growth performance of FUNAAB Alpha broiler chickens

Parameter	Control	Arginine	Arginine + Methionine	Arginine + Lysine	SEM	P-value
Initial weight (g/bird)	84.43	79.83	78.17	76.13	2.89	0.340
Final weight (g/bird)	1304.80	1414.10	1232.70	1282.90	73.3	0.449
Average weight gain (g/bird/day)	21.79	23.83	20.62	21.55	1.36	0.481
Total weight gain (g/bird/day)	1220.30	1334.30	1154.50	1206.80	76.0	0.481
Average feed intake (g/bird/day)	50.01	52.09	61.02	67.27	3.21	0.054
Total feed intake (g/bird)	2801.00	2917.00	3417.00	3767.00	179.00	0.054
Feed conversion ratio (FCR)	2.29^{b}	2.18^{b}	2.96^{a}	3.12^{a}	0.0831	0.003
Survivability (%)	92.86^{ab}	100.00^{a}	100.00^{a}	70.83^{b}	4.13	0.021

Means with different superscripts across the row are significantly different ($p<0.05$)

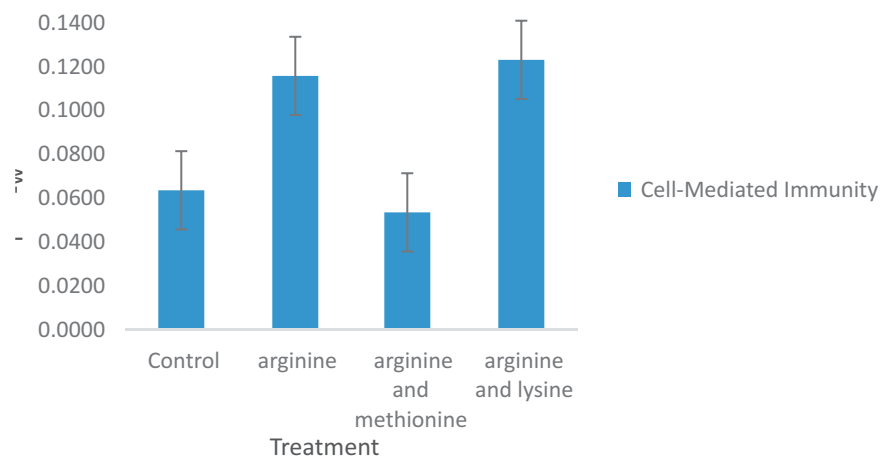

Figure. 3: Effect of *in ovo* injection of arginine, its combination with methionine and lysine on cell-mediated immunity of FUNAAB Alpha broiler chickens.

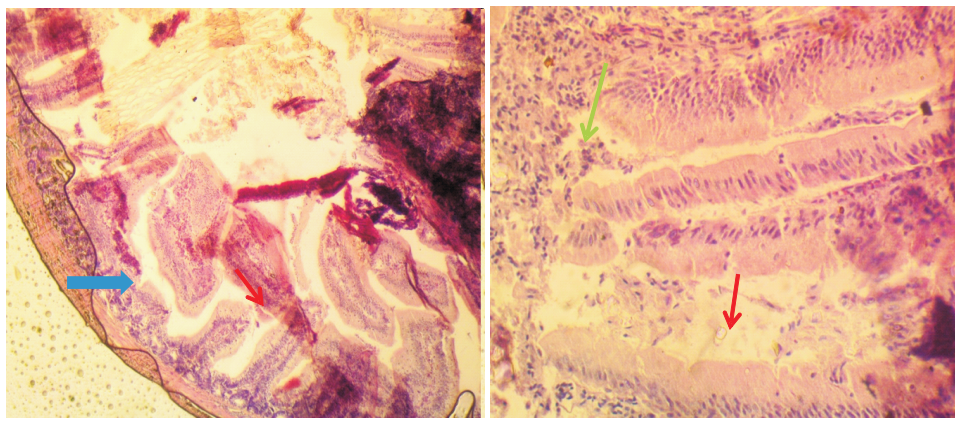

Plate 1: Duodenal histology of FUNAAB Alpha broiler chicken (Control)

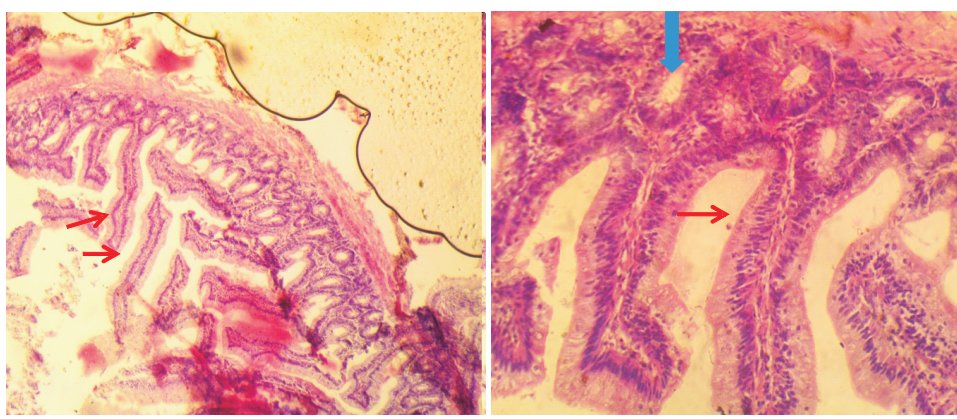

Plate 2: Duodenal histology of FUNAAB Alpha broiler chicken on *in ovo* injection of Arginine

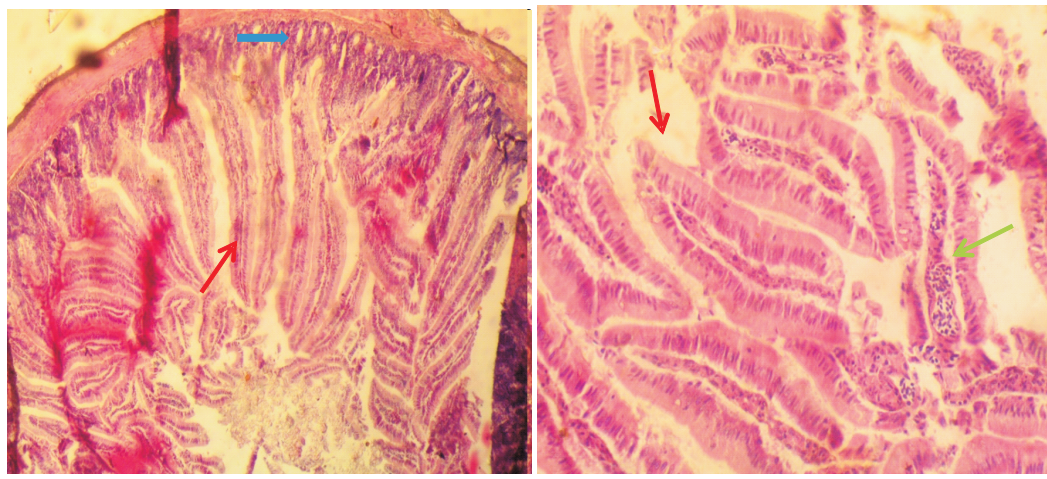

Plate 3: Duodenal histology of FUNAAB Alpha broiler chicken on *in ovo* injection of the combination of arginine and methionine

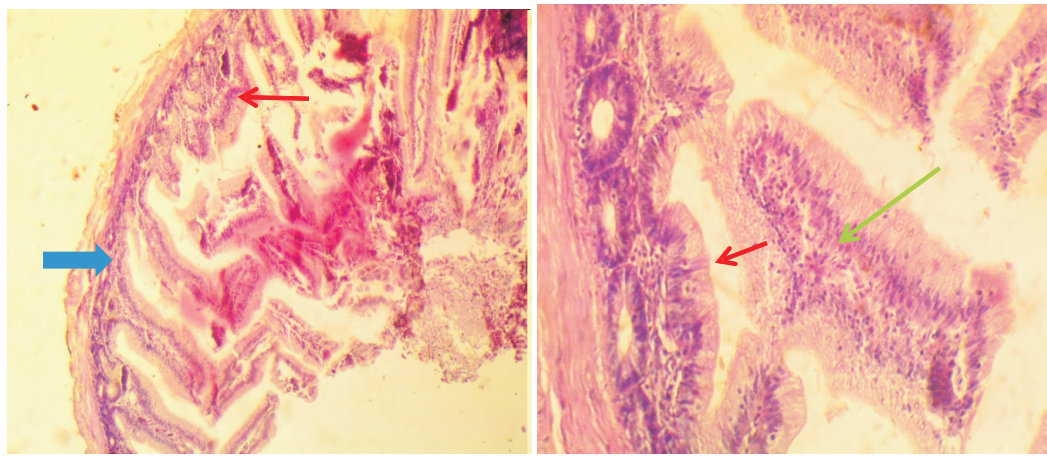

Plate 4: Duodenal histology of FUNAAB Alpha broiler chicken on *in ovo* injection of the combination of arginine and lysine

Table 3 : Effect of *in ovo* administration of amino acid on blood profile of FUNAAB Alpha broiler chickens at 3rd week of age

Parameter	Control	Arginine	Arginine + Methionine	Arginine + Lysine	SEM	P-value
Haematological Parameters						
Packed cell volume (%)	28.00	31.00	31.00	31.50	1.70	0.286
Haemoglobin (g/dl)	9.30	10.45	10.20	10.60	0.54	0.223
Red blood cell ($\times10^{12/L}$)	2.350	2.550	2.550	2.600	0.16	0.468
WBC ($\times10^{9/L}$)	11.20	11.75	11.90	13.20	1.01	0.640
Heterophil (%)	29.00	31.00	35.00	31.50	2.70	0.605
Lymphocyte (%)	70.00	67.50	63.50	65.50	2.40	0.398
Eosinophils (%)	0.50	0.00	0.50	0.50	0.44	0.898
Basophil (%)	0.50	1.00	0.00	1.00	0.31	0.275
Monocyte (%)	0.00	0.50	1.00	1.50	0.38	0.142
Serum biochemical parameters						
Total protein (g/dl)	4.10	4.65	5.20	3.45	0.78	0.563
Albumin (g/dl)	2.15	2.55	3.15	1.95	0.39	0.338
Globulin (g/dl)	1.95	2.10	2.05	1.50	0.43	0.757
Cholesterol (mg/dl)	121.75	114.95	103.60	120.10	7.82	0.425
Triglyceride (mg/dl)	123.05	119.05	90.25	104.20	8.53	0.148
Alkaline phosphatase (U/l)	34.00	29.00	30.50	31.00	4.98	0.886
Aspartate transaminase (U/l)	38.50	29.00	61.50	34.00	17.60	0.731
Alanine transaminase (U/l)	15.00	11.50	17.50	12.50	5.23	0.832

Discussion

The results revealed highest hatchability percentage of 70.27 in the control treatment followed by 51.35% in L-arginine-injected eggs and lowest hatchability percentage of 2.70 in L-methionine-injected eggs. This implies that the injection of methionine did not support the embryonic development unlike arginine which gave a better hatchability (Ohta *et al*., 2001). However, the low hatchability observed in methionine-injected eggs might be due to the concentration of the amino acid solution used, which leads to late embryonic mortality. Factors such as temperature instability during *in ovo* procedures and pH fluctuations in the eggs could also be attributed to the differences obtained in the hatchability of *in ovo* injected and un-injected eggs. Similarly, low hatchability result was reported by Coskun *et al.* (2014), when DL-methionine was injected into the fertile broilers eggs compared with the control. The outcomes of the first trial prompted the second trial where L-arginine was then combined with L-methionine as well as L-lysine. From Figure 2, the result showed highest hatchability percentage of 89.58 in the control. Unlike in trial 1, where arginine-injected eggs were next in percentage hatchability to the control, but the eggs injected with combination of arginine and Lysine nutrients recorded 39.29 hatchability. This was followed by those on *in ovo* injection of arginine and the lowest hatchability (21.43%) was recorded on hatching eggs injected with L-arginine and L-methionine combination. Coskun *et al.* (2014) reported that hatchability decreased when nutrient injected into the

amnion fluid is done with different injection depths. On the other hands, other researchers (Uni *et al*., 2005; Bottje *et al.*, 2010; Chamani *et al.*, 2012) signified that *in ovo* injection depths did not affect hatchability. Moreover, nutrients used for *in ovo* injection is an important factor for embryonic development and hatching performance. From the two trials in this study, it could be confirmed that embryogenesis process is aided by *in ovo* injection of L-arginine nutrient (Al-Daraji *et al*., 2012). Birds from eggs on *in ovo* injection of arginine had the best feed conversion ratio of 2.18 which is comparable to the value obtained in birds from eggs on *in ovo* injection of the combination of L-arginine and L-methionine (2.96) and those on the combination of arginine and lysine (3.12). This was corroborated by the finding of Al-Daraji *et al.* (2012). Survivability improved in chickens from eggs on *in ovo* injection of L-arginine and the combination of L-arginine and L-methionine thereby confirming the relative importance of the *in ovo* technique in boosting the bird's health (Sogunle *et al*., 2018).

Figure 3 revealed that chickens on *in ovo* injection of combined L-arginine and L-lysine had the highest cell-mediated immune response of 0.123, followed by those on L-arginine injection (0.116) and the least cell-mediated immune response (0.054) was from birds on the combination of L-arginine and L-methionine. The importance of amino acids to immunity has become apparent in recent years particularly in studies (Bilsborough, 2002; Brandtzaeg, 2009; 2011) which demonstrate not only the importance of Gut-Associated Lymphoid Tissue (GALT) and examine the immunomodulatory effects of specific amino acids on immunity. It has been recently reported (Wu *et al*., 1991) that T-cells, B-cells dendritic cells and macrophages express glutamate receptors (Sturgill *et al*., 2011) suggesting that glutamate likely has an important role in immune cell function. Wu *et al.* (2010) reported that the immune system is particularly sensitive to changes in arginine availability during early development. Birds have different levels of immune response to diverse external forces which was observed in this study. At day 7 in trial 2, effect of *in ovo* injection of amino acids on duodenal histology of indigenous shown in Plates 1, 2, 3 and 4 indicated variations in thickness and length of gastro intestinal tract and increase length of the villi in duodenum. *In ovo* injection of L-arginine showed the presence of few tall duodenal villi (red arrow in Plate 2). Also, duodenum of indigenous chicken on *in ovo* injection of the combination of arginine and methionine revealed several tall of villi and crypts which are slightly high. Meanwhile, that of control showed moderate amount of duodenal villi presence. Hu *et al.* (2013) reported dietary sources of amino acids were reported to influence villus height, villus width, crypt depth and mucosal thickness in gross histology of the intestine. *In ovo* feeding of amino acids may have been responsible for the development of gastro-intestinal tract of indigenous chicken in this study. Zhan *et al.* (2008) has earlier reported that dietary L-arginine supplementation supports the growth and development of the intestine and mucosal barrier in weanling piglets. Although, Al-Daraji *et al.* (2012) reported significant differences in the blood metabolites of chickens on in ovo injection of amino acids, this study showed no differences in the blood parameters determined.

Conclusion

The *in ovo* injection of the amino acids enhanced the gastrointestinal development of the FUNAAB-Alpha chicken. In addition, *in ovo* injection of L-arginine either singly or in combination with l-

methionine or l-lysine improved hatchability.

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