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Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits and post-hatch performance of broiler chickens in the tropics

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***Target audience**: Hatchery managers, poultry breeders, poultry farmers, policy makers*

Abstract

This study determined the effects of in ovo injection of inorganic salts of Zn, Cu and Mn on hatching traits and post-hatch performance of Arbor acre broiler chickens. A total of four hundred hatching eggs of Arbor acre strain of broiler chickens were sorted to give three hundred and twenty hatching eggs. On the 14th day of incubation, the eggs set were candled and 250 eggs (78.13% fertility) were fertile. The fertile eggs were redistributed into 4 treatments: Control (un-injected eggs), in ovo administration of 80 µg.egg^{-1} inorganic Zn, in ovo administration of 16 µg.egg^{-1} inorganic Cu and 0.3 µg.egg^{-1} inorganic Mn) each consisting of 62 eggs. The in ovo injection was carried out on the 18th day of incubation. After hatching, the birds were managed intensively for a period of 6 weeks in a deep litter housing. Data were subjected to Completely Randomized Design. Results revealed hatchability ($p<0.05$) percentage of 74.71% in the Control (un-injected eggs), followed by similar value of 63.00% each in birds from Zn and Cu-injected eggs. The chick weight was significantly ($p<0.05$) highest (68.03 g) in birds from the Control (un-injected eggs), followed by (66.15 and 67.90 g) in birds from Zn and Cu-injected eggs, respectively and lowest (60.90%) in birds from Mn-injected eggs. On day 7 of age, birds from Mn-injected eggs had significantly ($p<0.05$) highest proportion of heart (0.80%) while 0.78 and 0.75% were obtained in birds from the Control (un-injected eggs) and Zn-injected eggs, respectively with the lowest proportion of 0.61% in birds from Cu-injected eggs. Birds from Cu-injected eggs at day 42 of age had highest (11.90x10^9/L) White blood cell count. The mid-shaft width of birds from Zn-injected eggs recorded significantly ($p<0.05$) highest value (10.23 mm). The study concluded that in ovo injection of inorganic salts of Zn and Cu improved hatchability comparably to the control treatment. In ovo injection of Zn salt improved growth of the heart (at 7 days of age), duodenum percent (at 42 days of age) as well as tibia mid-shaft width.

***Keywords**: In-ovo injection, Zinc, Copper, Manganese, performance, Arbor acre broiler chicken, gut morphology*

Description of problem

Due to the economic importance of broiler chickens in the production of cheap and readily available protein in human diets, understanding factors that can enhance its production is of significance. Under practical conditions, many birds have access to feed only 36 to 48 hours after hatching, and during this time body weight decreases, and intestine and muscle development are retarded. In order to overcome these limitations, *in ovo* injection which is a continuous feeding process was developed to supply nutrients to the developing embryo, feed and water to the newly-hatched chick within the hatcher.

It is worthy of note that micro-minerals that are important to bone formation and strength include Zn, Mn and Cu are greatly reduced in concentration in the egg by the 17^{th} day of incubation (31). Due to the trace mineral deficiencies, improper bone formation, malfunctioning of the basic connective tissues causes leg problems often predominant in broiler chickens, reduces immunity or post-hatch growth performance of the embryo.

Zinc is an essential trace mineral for birds, functioning elaborately in enzyme systems which are also involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions. Zinc is required for normal growth, reproduction, and glandular development of birds (2). Zn deficiency can result in poor growth and abnormal bone development in chicks. The involutions of the thymus and spleen weights are characterized by zinc deficiency and primarily the absence of white blood cells (28). Inadequate intracellular concentration of zinc also causes damage to the lymphocyte function that is responsible for the ability of T-and B- cell proliferation.

Copper is one of the most critical trace minerals as it plays vital role in hemoglobin synthesis and is associated with many enzymes (9). Copper deficiency in growing animals impairs the integrity and functioning of connective tissue, most notably the collagen and elastin network in aorta. However, Copper is also related to the synthesis of hemoglobin, erythrocuprein and other plasma proteins necessary for lipid metabolism, hepatic lipogenic enzyme activities, and the maturation of hematopoietic cells (1;7). Cu was found to enhance the immune response of broiler chickens on *in ovo* administration of 8 μg.egg-1 of inorganic Cu in $CuSO_4$ (10).

Manganese (Mn), which is the fifth most abundant element on earth, is one of the trace elements and is known to be essential in animals (25). In poultry nutrition, Mn is essential for embryonic development, normal growth of the body, bones and reproduction. It is also responsible for carbohydrate and lipid metabolism (26). In another report (16), Mn inadequacies can lead to the malformation of the epiphyseal plate of the tibia. Mn is added to the diet in the sulphate form, which is typically used as the standard in nutritional studies. The dietary Mn requirement for poultry may no longer be sufficient to maintain the optimal performance of birds due to the considerable improvement in their genetic potential. Therefore, these micronutrients (Zn, Mn and Cu) play vital roles in poultry production; and deficiency in any will reduce hatchability, cause poor body conformation and immune system. This study thereby investigated the effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits and post-hatch performance of Arbor acre strain of broiler chickens in tropical environment.

Materials and Methods

Experimental Site

The hatching study was carried out at the Hatchery Unit of the College of Animal Science and Livestock Production while the field trial was conducted at the Poultry unit of Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. The experimental site lies within latitude 7°10"N and longitude 3°2"E. It has an altitude of 76mm and is located within the derived savannah zone of South-Western

Nigeria. It has a humid climate with mean annual rainfall of 1037mm and temperature of about 34.7^{0}C (Federal University of Agriculture, Abeokuta Meteorological Centre).

Source of Hatching Eggs

A total of four hundred hatching eggs of Arbor acre strain of broiler chickens were sorted to give three hundred and twenty hatching eggs. These were procured from a reliable commercial Breeder Farm in Ibadan, Oyo State, Nigeria.

Management of Hatching Eggs in the Incubator

The hatching eggs were fumigated, weighed and arranged in a setter tray within the incubator. On the 14th day of incubation, the eggs were candled and eggs showing viable embryo were distributed into four groups including control and *in ovo* supplemented groups (Table 1). The pointed ends of the eggs were positioned downward. Eggs were turned automatically on hourly basis during the incubation period; while temperature and humidity of the incubator were monitored. Adequate ventilation was also maintained. Within the incubator, hatching eggs were managed under an optimum temperature and humidity of 37.8ºC and 60%, respectively.

On the 18th day of incubation, the eggs were carefully transferred from the setter compartment to the hatcher compartment for hatching on the 21st day of incubation.

Table 1: Groups of eggs for *in ovo* injection

Treatment groups	*In ovo* injection
Group I	Control
Group II	*In ovo* supplementation with 80 µg.egg^{-1} of inorganic Zinc (Zn Sulphate 351.80 µg. 0.5 ml^{-1}deionized water)
Group III	*In ovo* supplementation with 16 µg.egg^{-1}of inorganic Copper (Cu Sulphate 62.87 µg. 0.5 ml^{-1}deionized water)
Group IV	*In ovo* supplementation with 0.3 µg.egg^{-1} of inorganic Manganese (Mn Sulphate 0.657 µg. 0.5 ml^{-1}deionized water)

Procedure for *in ovo* injection of inorganic salts

At 14th day of incubation, the eggs were candled and two hundred and fifty eggs were confirmed with viable embryo. These 250 fertile eggs were divided into four *in ovo* groups. On 18th day of embryonic age, two hundred and fifth six eggs with viable embryos were divided into four treatments of eight replicates each with 64 eggs per treatment and 8 eggs per replicate. These eggs were injected with the inorganic salts into the amnion using a 24-gauge hypodermic needle of 25 mm long (3). The *in ovo* injection for each treatment was completed within 10 minutes of taking out the eggs from the incubator. Before injection, the site was sterilized using ethanol and the injection was administered at the broad end of the egg. After *in ovo* feeding, the injected site was sealed with candle wax and the eggs were transferred to hatching compartment.

Post-hatch chick Management

After hatching, the hatched chicks (113 chicks total i.e. 56.5% hatchability) from the treatments were reared on a deep litter housing system**.** The hatched chicks included: 38 chicks (74.71% hatchability) for the Control, 31 chicks (63% hatchability) from Zn-injected eggs, 31 chicks (63% hatchability) from Cu-injected eggs and 13 chicks (25% hatchability) from Mn-injected eggs. The birds were then replicated thrice except for birds from Mn-injected eggs that were removed (due to poor hatchability) from the treatment after the first week of rearing. Floor was covered with litters (wood shaving) up to 5 cm thickness, cardboard was used for partitioning of the pens and heat sources were provided using incandescent bulbs and charcoal pots. Feeders and drinkers were under uniform and standard management condition. Standard broiler pre-starter (0-7 days), starter (7-21 days) and finisher (21-42 days) diets were supplied to the birds (Table 2). Feed and drinking water were provided *ad libitum*. The experiment lasted for 42 days.

Table 2. Ingredient and nutrient composition (%) of experimental diets

Ingredients	Pre-starter (0-7 days)	Starter (1-3 wk)	Finisher (3-6 wk)
Maize	57.00	58.60	62.50
Soybean meal	37.00	36.10	31.50
Fat/oil (soy bean oil)	1.87	1.65	2.20
Limestone	1.00	1.00	1.10
Dicalcium Phosphate	1.75	1.75	1.75
Salt (NaCl)	0.35	0.35	0.35
Lysine	0.40	0.10	0.12
Methionine	0.20	0.20	0.20
Threonine	0.18	0.00	0.00
*Vitamin and Minerals premix	0.25	0.25	0.28
Total	100.00	100.00	100.00
Calculated Analysis			
ME (Kcal/kg	2995.50	2991.50	3047.75
Crude protein (%)	22.51	21.89	20.07
Lysine (%)	1.52	1.26	1.15
Methionine (%)	0.55	0.51	0.44
Threonine (%)	0.98	0.78	0.72
Tryptophan (%)	0.23	0.23	0.21
Valine (%)	0.89	0.88	0.80
Arginine (%)	1.37	1.35	1.21
Ca (%)	1.00	1.00	1.00
P, avail. (%)	0.45	0.45	0.45
Zn (ppm)	190.30	90.30	88.1
Cu (ppm)	29.30	14.30	13.8

*Vitamin and mineral premix 0.1%, Vitamin Premix 0.1%, B- Complex 0.02%, Choline 0.05% and Salt 0.3% Trace mineral Premix supplied $mg.kg^{-1}$diet: Mg 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit. A, 8250 IU; Vit D3, 1200 ICU; Vit. K, 1mg; Vit E, 40 IU; Vit.B1, 2mg; Vit.B2. 4mg; Vit B12; 10mcg; niacin; 60mg; pantothenic acid; 10mg; choline, 500m

Table 3: Effect of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits of broiler chickens

	***In ovo* injection of inorganic mineral salts**					
Parameters	**Control**	**Zn**	**Cu**	**Mn**	**SEM**	**P-value**
Egg Weight (g)	68.03	66.15	67.90	60.10	1.79	0.112
Hatchability (%)	74.71^{a}	63.00^{ab}	63.00^{ab}	25.00^{b}	8.97	0.042
Chick weight (g)	50.50^{a}	42.40^{b}	40.87^{b}	40.75^{b}	3.35	0.011
Egg : Chick	0.74	0.64	0.60	0.59	0.21	0.057

^{ab} Means with different superscripts on the same row are significantly ($p<0.05$) different.

Table 4: Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on organs development and intestinal morphology of broiler chickens at 7 and 42 days of age

	***In ovo* injection of inorganic mineral salts**					
Parameters	**Control**	**Zn**	**Cu**	**Mn**	**SEM**	**P-Value**
At 7th day of age						
Live weight (LW) (g/bird)	65.50	68.00	46.50	62.00	6.47	0.228
Liver (%)	3.01	3.05	0.50	2.79	1.91	0.751
Heart (%)	0.78^{a}	0.75^{a}	0.01^{b}	0.81^{a}	0.07	0.003
Proventriculus (%)	0.33	0.75	0.01	0.81	0.17	0.082
Gizzard (%)	0.09	0.13	0.01	0.03	0.08	0.729
Duodenum length (cm/100g LW)	18.83	18.62	22.94	18.23	1.80	0.343
Jejunum length (cm/100g LW)	36.30^{b}	38.29^{b}	48.57^{a}	38.46^{b}	1.37	0.010
Ileum length (cm/100g LW)	35.12	35.76	42.31	33.70	3.59	0.436
Caecum length (cm/100g LW)	15.48	16.24	18.45	14.09	1.51	0.353
Colon length (cm/100g LW)	6.48	4.37	6.72	5.35	1.44	0.665
At 42nd day of age						
Live weight (LW) (g/bird)	1570.00	1641.70	1679.20	-	70.20	0.526
Lung (%)	0.53	0.49	0.55	-	0.06	0.815
Liver (%)	2.17	1.89	2.15	-	0.70	0.680
Proventriculus (%)	0.41	0.37	0.33	-	0.05	0.470
Gizzard (%)	1.89	1.65	1.70	-	0.13	0.402
Bursa (%)	0.13	0.13	0.01	-	0.09	0.572
Thymus (%)	0.46	0.55	0.45	-	0.15	0.873
Pancreas (%)	0.27	0.31	0.30	-	0.06	0.913
Duodenum length (cm/100g LW)	2.01	1.88	1.61	-	0.13	0.179
Duodenum (%)	0.88^{ab}	1.07^{a}	0.68^{b}	-	0.07	0.048
Jejunum length (cm/100g LW)	4.55	4.44	2.63	-	0.65	0.169
Jejunum (%)	2.15	1.91	1.71	-	0.45	0.779
Ileum length (cm/100g LW)	4.85	4.70	3.64	-	0.30	0.072
Ileum (%)	2.25	1.98	1.79	-	0.45	0.752
Caecum length (cm/100g LW)	2.20	1.99	1.53	-	0.16	0.065
Caecum (%)	0.62	0.61	0.48	-	0.07	0.309
Colon length (cm/100g LW)	0.34	0.43	0.24	-	0.07	0.298

^{a,b} Means with different superscripts on the same row are significantly ($p<0.05$) different.

Data Collection

Hatching Traits

Egg Weight: measurement for egg weight was taken before incubation with the aid of a sensitive scale (CAMRY®, SF-400A).

Chick Weight: chick weight was determined after hatching. This was also measured with the use of a sensitive scale (CAMRY®, SF-400A).

Egg to Chick Ratio: the Egg-to-chick ratio was deduced after hatching with the relation below:

Egg to Chick = Egg weight (g)/ chick weight (g)

Percentage Hatchability: this was calculated by dividing the quantity of hatched chicks by the quantity of viable embryo expressed as a percentage.

$$\% \text{ Hatchability} = \frac{\text{No of hatched chicks}}{\text{No of viable embryo}} \times 100$$

Assessing Gastro- intestinal tract and Organs Development of the birds

On 7^{th} and 42^{nd} day post-hatch, two birds from each replicate were slaughtered using neck slitting by cervical dislocation for GIT and organs developmental studies. GIT morphology was done by measuring the gizzard weight, proventriculus, liver as well as the weight and length of the duodenum, jejunum, ileum, caecum and colon. The weight measurement was carried out with the aid of a sensitive scale (Manlloro® digital pocket scale) while the lengths were measured with the aid of centimeter ruler. The lengths of the intestinal segments measured were expressed as cm/100 g of the live weight of the birds.

Determination of Haematological parameters

On 7^{th} and 42^{nd} day post-hatch, two birds of average weight were selected from each replicate for blood collection; 2ml of blood sample was collected from each bird using 5ml hypodermic needle and syringe. The blood samples collected was placed in EDTA bottle for the determination of haematological parameters (Packed cell volume, Hemoglobin, Red blood cell, White blood cell and White blood differential counts) according to standard procedures (13).

Growth Performance

Growth performance was measured using the following indices:

Feed intake: this was measured weekly and recorded for each replicate. Feed leftover was subtracted from the amount of feed offered to the birds weekly to determine the feed intake. Average feed consumed by a bird was calculated by the formula:

Feed intake =*total feed offered – total left-over feed*

$$\text{Avenge feed intake (g/bird)} = \frac{\textit{Feed intake}}{\textit{Number of birds per replicate}}$$

Body weight gain: average body weight per bird was calculated by deducting the initial body weight from the final body weight and dividing the resulting value by the number of birds per replicate.

$$\text{Average body weight gain (g/bird)} = \frac{\text{Final weight (g) – initial weight (g)}}{\text{Number of birds per replicate}}$$

Feed conversion ratio (FCR): The FCR was calculated by dividing the feed intake by the weight gain.

$$\text{FCR} = \frac{\textit{Total feed consumed (g)}}{\textit{Body weight gain (g)}}$$

Table 5: Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on haematological parameters of broiler chickens at 7th and 42nd days of age

	In ovo injection of inorganic mineral salts					
Parameters	**Control**	**Zn**	**Cu**	**Mn**	**SEM**	**P-Value**
At 7th day of age						
Packed Cell Volume (%)	26.00	22.50	22.50	24.50	2.63	0.751
Hemoglobin (g/dl)	8.75	7.55	7.55	8.25	0.87	0.730
Red Blood Cell($x10^{12}$/L)	2.25	1.95	1.90	2.05	0.25	0.769
White Blood Cell ($x10^9$/L)	10.30	11.35	11.60	10.00	0.58	0.284
Heterophil (%)	38.00	32.00	34.00	30.00	5.74	0.790
Lymphocyte (%)	60.00	68.00	65.00	68.00	5.70	0.738
Eosinophils (%)	0.50	0.01	0.50	0.01	0.35	0.615
Basophil (%)	1.00^a	0.00^b	0.00^b	1.00^a	0.00	<0.001
Monocyte (%)	0.50	0.00	0.50	1.00	0.35	0.381
MCV	115.75	115.60	118.70	119.50	2.03	0.488
MCH	38.90	38.80	39.90	40.25	0.69	0.450
MCHC	33.65	33.55	33.60	33.70	0.37	0.992
At 42nd day of age						
Packed Cell Volume (%)	33.50	30.00	34.00	-	32.50	0.271
Hemoglobin (g/dl)	10.68	13.00	11.30	-	0.89	0.162
Red Blood Cell ($x10^{12}$/L)	2.65	3.35	2.85	-	0.23	0.172
White Blood Cell ($x10^9$/L)	9.80^b	10.00^{ab}	11.90^a	-	0.41	0.030
Heterophil (%)	29.25	36.00	36.00	-	2.83	0.195
Lymphocyte (%)	71.25	63.50	63.50	-	2.05	0.050
Eosinophils (%)	0.50	0.00	0.00	-	0.29	0.363
Basophil (%)	0.75	0.50	0.00	-	0.51	0.595
Monocyte (%)	0.75	0.00	0.50	-	0.51	0.595
MCV	119.80	119.55	119.30	-	0.65	0.853
MCH	40.28	40.15	39.65	-	0.25	0.279
MCHC	33.63	33.60	33.20	-	0.16	0.231

^{a,b} Means with different superscripts on the same row are significantly ($p<0.05$) different.
MCV= Mean Corpuscular Volume
MCH= Mean Corpuscular Hemoglobin
MCHC= Mean Corpuscular Hemoglobin Concentration

Table 6: Effect of *in ovo* injection of inorganic salts of Zn and Cu on growth performance of broiler chickens

	In ovo injection of inorganic mineral salts				
Parameters (g/bird)	**Control**	**Zn**	**Cu**	**SEM**	**P-value**
Initial weight	106.38	110.88	93.17	4.82	0.143
Final weight	1570.00	1641.70	1679.00	70.20	0.526
Daily weight gain	41.82	43.74	45.31	2.00	0.475
Daily feed intake	90.04	92.03	92.67	3.37	0.261
Feed conversion ratio (FCR)	2.15	2.11	2.06	0.16	0.713

Table 7: Effects of *in ovo* injection of inorganic salts of Zn and Cu on Tibia morphometry of broiler chickens

	In ovo injection of inorganic mineral salts				
Parameters (mm)	**Control**	**Zn**	**Cu**	**SEM**	**P-value**
Tibia Width	5.75	6.15	4.90	0.43	0.246
Tibia Length	67.08	67.88	67.55	1.03	0.842
Proximal Width	20.31	18.55	18.56	0.70	0.168
Distal Width	18.12	17.16	17.17	0.63	0.459
Mid-shaft Width	8.40^{b}	10.23^{a}	7.88^{b}	0.36	0.017

^{ab} Means with different superscripts on the same row are significantly (p<0.05) different.

Evaluation of Tibia morphometry

At the 42nd day of age, two birds from each replicate (of average weight) were sacrificed through neck slitting, the left tibia from the birds slaughtered were collected and their adhering muscles along with connective tissues were carefully detached by hand and dipped into boiling water for 5 minutes to eliminate any residual soft tissues. Measurement was done on the length, proximal and distal width, as well as the mid shaft width of the tibia using Vernier caliper expressed in mm.

Statistical analysis

Data collected during the experiment were subjected to One-way Analysis of variance in a completely randomized design. Significantly (P<0.05) different means among variables were separated using Tukey test contained in Minitab® version 17.1.0 (18).

Results

Effect of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits of broiler chickens

In Table 3, the effect of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits of broiler chickens is shown. Hatchability percentage and chick weight were significantly (p<0.05) different among treatments. Hatchability percentage revealed significantly (p<0.05) highest (74.71%) in the control; followed by similar value of (63.00%) each in treatments from Zn and Cu-injected eggs with the lowest (p<0.05) of (25.00%) obtained in treatments from Mn-injected eggs. Chick weight was significantly (p<0.05) higher (50.50 g) in treatments on control and lowest (42.40, 40.87 and 40.75 g) in treatments from injected eggs of Zn, Cu and Mn groups, respectively.

It was observed that *in ovo* injection of Mn resulted to poor hatchability and as a result; chicks from the Mn injected eggs were only managed for 7 days with the other *in ovo* treatments, while the few birds were sacrificed to evaluate the gastro-intestinal development at 7 days of age.

Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on organs development and GIT morphology of broiler chickens at 7th and 42nd days of age

The effect of *in ovo* injection of inorganic salts of Zn, Cu and Mn on organs development and GIT morphology of broiler chickens at 7 days of age is presented in Table 4. Heart and jejunum length were significantly (p<0.05) different among the treatments. Birds in control, and those from injected eggs of Zinc and Manganese had significantly (p<0.05) higher values (0.78, 0.75 and 0.81%, respectively) for heart relative to (0.01) in birds from Copper-injected eggs. Jejunum length was significantly higher (48.57 cm/100g LW) in birds from eggs injected with Cu.

The effects of *in ovo* injection of inorganic salts of Zn and Cu on organs development and GIT of broiler chickens at 42 days of age revealed that the duodenum was significantly ($p<0.05$) highest (1.02%) in birds from Zn-injected eggs and lowest (0.68%) in birds from Cu-injected eggs.

Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on haematological parameters of broiler chickens at 7 days of age

Table 5 shows the effects of *in ovo* injection of inorganic salts on haematological parameters of broiler chickens at 7 days of age. Basophils were significantly ($p<0.05$) different across the treatments. The highest (1.00 %) basophils were recorded in the control and birds from Mn-injected eggs, while the lowest (0.00%) values were recorded in birds from Zn- and Cu-injected eggs.

The effects of *in ovo* injection of inorganic salts of Zn and Cu on haematological parameters of broiler chickens at 42 days of age shows that white blood cell (WBC) had significant ($p<0.05$) variations across the treatments. The highest WBC of 11.90×10^9/L was recorded in birds from Cu-injected eggs, while lowest value of 9.80×10^9/L was recorded in the control.

Effect of *in ovo* injection of inorganic salts of Zn and Cu on growth performance of broiler chickens

The effects of *in ovo* injection of inorganic salts of Zn and Cu on growth performance of broiler chickens are presented in Table 6. There were no significant variations in all the growth performance parameters measured.

Effect of *in ovo* injection of inorganic salts of Zn and Cu on bone morphometry of broiler chickens

Table 7 shows the effects of *in ovo* injection of inorganic salts of Zn and Cu on tibia morphometry of broiler chickens. Mid-shaft width recorded significant ($p<0.05$) variations among treatment means. Birds from Zn-injected eggs recorded significantly ($p<0.05$) higher mid-shaft width of 10.23 mm while lower values (8.40 and 7.88 mm) were recorded in the control and birds from Cu-injected egg.

Discussion

Significantly highest hatchability was recorded in eggs under the control treatment with comparably improved hatchability noted in eggs injected inorganic salts of Zn and Cu relative to Mn-injected eggs. This implies that *in ovo* injections of Zn and Cu enhances the metabolic processes of embryogenesis owning to their roles in body physiological functions. Zinc is distributed throughout the body and plays critical roles in improving reproduction, development of blood cells, immune system function, and bone development (1; 11). The relatively improved hatchability in Zn-injected eggs is similar to the findings (2; 24) who affirmed zinc as a crucial trace minerals for embryonic development. Copper is a component of blood proteins (cytochrome oxidase) of tissues that are fundamental for metabolic and respiratory functions (15). However, significantly poorer hatchability observed in birds from Mn-injected eggs indicated that injection of Mn did not support embryogenesis thus resulting in high embryonic mortality. This can be adduced to the quantity of Mn administered *in ovo* which may be toxic to the embryo during peri-natal period. Toxicity of Mn interfering with the availability of other minerals has been reported (29). Variation in the hatchability can also be attributed to other factors as reported in previous studies to include: injection volume and concentration (8) injection time and depth (20). In this study, hatchability was significantly different among the *in ovo* treatment groups, which is consistent with previous reports (17) that *in ovo* injection decreased hatchability while another authors (4) found that *in ovo* injection increased hatchability.

The digestive system of chicks undergoes a number of morphological changes such as increase in length and density of intestinal villi (30). Supplementation of trace minerals such as Zinc in diets improves intestinal morphology by increasing the villus height and crypt depth in poultry (5). Improvement observed in heart (at 7 days of age) and duodenum (at 42 days of age) percentages of birds resulting from *in ovo* injection of Zn. This can be attributed to the biological functions of zinc as an essential trace element required for growth, bone development, enzyme structure and function, and appetite for avian species. Zinc is responsible in numerous cellular metabolisms and is required for catalytic activity of several enzymes (22) which promotes the secretion of gastro-intestinal hormones. This result on the heart percent corroborated the findings (24) that showed significantly higher proportion of heart at day 7 of age in broiler chicken resulting from *in ovo* administration of inorganic salts of zinc, copper and combination of copper, zinc and selenium. Higher jejunum length (at day 7 of age) was observed in broiler chickens resulting from *in ovo* injection of Cu. Cu has been reported to possess antimicrobial properties and improves intestinal growth and health with significant influence on intestinal microbiology (6). The results on the influence of *in ovo* injection of the inorganic salts on organ development and gut morphology of the broiler chickens at days 7 and 42 are similar to the works (27) which concluded that *in ovo* feeding resulted to improvement in the development of gastrointestinal tracts in broiler chickens.

Literature is limiting on the influence of *in ovo* feeding of in organic salts on haematological indices of broilers chickens. However, at 7 days of age, higher basophil was observed in birds under the control as well as birds resulting from *in ovo* injection of Mn. Also, the white blood cell count was higher in birds resulting from *in ovo* injection of Cu (at 42^{nd} day of age) relative to the other treatment groups. This indicated that the *in ovo* administration of Cu has the potential to improve the immune system of the broiler chickens. High WBC counts are an indication of increased synthesis of antibodies which produces a high level of disease resistance (23). Copper is also related to the synthesis of hemoglobin, erythrocuprein and other plasma proteins necessary for lipid metabolism, hepatic lipogenic enzyme activities, and the maturation of hematopoietic cells (7). Cu was found to enhance the immune response of broiler chickens on *in ovo* administration of 8 μg.egg-1 of inorganic Cu (10). Values recorded for the haematological parameters in the broiler chickens at 7^{th} and 42^{nd} days of age were consistent with the normal reference ranges documented (21) for chickens.

In ovo administration of inorganic salts of zinc and copper did not significantly influenced any of the growth performance indices. This outcome can be adduced to the difference in the strain response to *in ovo* injection of inorganic salt as a contrary observation was reported (24) to improve final weight and weight gain due to *in ovo* administration of Zn, Se, Cu and their combination in fertile egg of Cobb broiler chickens. However, a numerically better feed conversion ratio was recorded in birds resulting from eggs injected Cu. This is an indication though not significant that birds from Cu-injected eggs utilized nutrient efficiently. Similar report was noted (19) when trace minerals of Cu, Zn and Mn were supplemented in the diets of broiler chickens.

The bone morphometric parameters measured in this study revealed that *in ovo* administration of Zn increased the mid-shaft width. Zinc has a stimulatory effect on bone formation and mineralization *in vivo* and *vitro* (12). Its deficiency causes skeletal malformations, poor bone mineralization and immunological dysfunctions (14).

Conclusion and Applications

1. It could be concluded that *in ovo* injection of inorganic salts Zn and Cu improved hatchability comparably to the control treatment while *in ovo* injection of Mn depressed hatchability as well as the chick weight. Chicks resulting from *in ovo* injection of Zn salt had improved growth of the heart (at 7 days of age), duodenum percent (at 42 days of age) as well as tibia mid-shaft width.
2. *In ovo* administration of inorganic salts (Zn, Cu and Mn) did not pose any deleterious effects on the health status of the broiler chickens at 7th and 42nd days of age.
3. *In ovo* injection of Zn and Cu is therefore recommended for broiler chicken production in the tropical environment.

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