sciendo

DOI: 10.2478/fv-2023-0001

FOLIA VETERINARIA, 67, 1: 1—14, 2023

EFFECTS OF *IN OVO* INJECTION OF INORGANIC SALTS OF ZINC AND COPPER ON PERFORMANCE AND SERUM BIOCHEMICAL INDICES OF TWO STRAINS OF BROILER CHICKENS

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ABSTRACT

This study was composed of two experiments which investigated the response of two strains (Arbor Acre and Cobb 500, respectively) of broiler chickens to *in ovo* injection of inorganic salts of zinc, copper and their combination. A total of 300 hatching eggs [only 148 (59.20 %) and 232 (90.27 %), respectively, were fertile] each of Arbor Acre and Cobb 500 strains of broiler chickens were used in both experiments. These eggs were distributed into four treatments: control, *in ovo* inorganic Zn (80 µg.egg^{-1}), Cu (16 µg.egg^{-1}) and combined Zn and Cu (80 µg.egg^{-1} Zn and 16 µg.egg^{-1} Cu). The data obtained in both experiments were subjected to Completely Randomized Design (CRD) at the 5 % probability level. The results showed increased hatchability ($P < 0.05$) in eggs injected with the combination of inorganic salts of Zn and Cu in Experiment I and daily intake was influenced in both experiments. The carcass traits, organ development and gut morphometry were not significantly influenced by the treatment groups. The total serum protein and albumin of the birds were significantly ($P < 0.05$) increased by *in ovo* injection of inorganic salts of Zn and Cu at day 49 in the Experiment I. The study concluded that *in ovo* injection of inorganic salts of Zn at 80 µg.egg^{-1} and/or Cu at 16 µg.egg^{-1} could be adopted to increase feed intake with: attendant enhanced growth, enhanced immune response, increased albumin and total protein contents of blood serum in the strains of broiler chickens used.

Key words: Arbor Acre; Cobb 500; Cu; *in ovo* injection; Zn

INTRODUCTION

Most research on poultry are carried out post-hatch with the objectives of improving rearing or health conditions and optimisation of nutrition for an enhanced productivity. On the other hands, recent advances in technology and techniques as well as the possibilities associated with egg micro-manipulation have opened up completely new research on the birds' embryos. The research documents

the impact of various biologically important compounds including: amino acids and their derivatives, hormones, antibiotics, and inorganic salts on the development and biochemical changes of embryos, hatchability, as well as post-hatch growth performance of chicks [34, 40].

The nutritional management in young broiler chicks is essential for good performance during the growing period, because the digestive system develops mainly during the initial growth phase, followed by muscular and skeletal growth. However, the short lifespan of broiler chickens, discourages enough time for compensatory growth [6, 16, 22]. It is pertinent to stimulate the initial growth of the birds during the first days of age to achieve maximum growth rates; hence, the relevance of *in ovo* feeding. In a study [9] of *in ovo* nutrition, it was reported to improve: the digestive capacity, increase growth rate, and feed efficiency, reduce post-hatch mortality and morbidity, improve immune response to enteric antigens, reduce the incidence of developmental skeletal disorders, and increase muscle development and meat yield. The late term embryo obtains nutrients via the amnion fluid [15], and delivery of extra nutrients to this area compensated for any energy deficiencies during hatch.

The nutritional deficiencies associated with trace minerals such as Cu and Zn have pointed to the significant roles of these minerals in maintaining normal physiological processes in poultry production [17]. In recent time, biological assay methods clarified the significance and importance of mineral elements for human and animal nutrition and modern analytical techniques led to the detection of trace elements as essential nutrients and this is still an active area of current research. Zinc is essential for a variety of biochemical processes. Dietary Zn supplementation enhances the expression levels of metallothionein (MT) and copper- and Zn-containing superoxide dismutase as free radical scavengers in the tissues of broilers [23, 33]. In a study [39], involving *in ovo* injection of a solution containing Zn, improved the mechanical properties of the bones of chicks at 3 and 14 days of age. The authors [4] injected a mixture of Zn and other trace elements into the amniotic cavity of the 18 days old embryos, and found that the enrichment modulated the cell-mediated immunity of the resulting broiler chicks. On the other hands, it was posited [29] that copper is important in promoting efficient growth and health in poultry due to its roles in diverse biochemical processes with the tendency to improve nutrients transport and overall productive performance. Broiler chickens spend about 38 % of their entire lifespan inside the egg as embryos; thus embryogenesis is quite a critical period of growth and development in chickens [3, 27].

The Arbor Acre strain of broiler chickens is being steadily improved to ensure all products consistently add value to customer operations through established breed selection processes that use both traditional scientific techniques and the latest in technology (https://www.eu.aviagen.com). On the other hands, the Cobb 500 strain of broiler chicken is a flexible broiler that is arguably the world's most productive efficient line of meat chickens. It has: a very low feed conversion ratio, converts cheap low-density feed very well, has consistent performance across climates, produces optimally with the best uniformity, and it has consistent processing results including: white feather, yellow skin as carcass, and about 96 % liveability (https://www.cobb-vantress.com/en_US/products). The benefits in terms of meat yield of these products could not be undermined in meeting the protein needs of the teeming population.

The authors [39] stated that the embryonic nutrient reserves are insufficient and might be depleted in the embryonic period because of the increased metabolic rate of the embryos of today. Therefore, *in ovo* technique provides a practical means to safely introduce exogenous nutrients into developing embryos [5, 11, 20] to salvage the problem associated with nutrient depletion during embryonic development. This study investigated the response of two strains of broiler chickens to *in ovo* injection of inorganic salts of copper, zinc and their combination.

MATERIALS AND METHODS

Description of the experiments

This study comprised of two experiments which were undertaken concurrently. In Experiment I, the response of Arbor Acre strain of broiler chickens to *in ovo* injection of inorganic salts of zinc and copper and their combination was studied while the response of the Cobb 500 strain of broiler chickens to *in ovo* injection of inorganic salts of zinc and copper and their combination was studied in Experiment II.

Ethical consideration

The study protocol was approved by Animal Care and Use Review Committee (AC&URC) of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria [13].

Experimental site

The hatchery phase of the experiments was carried out at the hatchery of the College of Animal Science and Livestock Production, while the field trial was undertaken at the poultry unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Nigeria located at Latitude 7° 15'N, Longitude 3° 26'E.

Source and preparation of test ingredients

Inorganic salts of zinc and copper used for this study were obtained from a reputable veterinary store within Ogun State. Thereafter, the compounds were dissolved in deionised water at 80 µg of inorganic salt of zinc ($ZnSO_4.7H_2O$) and 16 µg of inorganic salt of copper ($CuSO_4.5H_2O$) into 100 ml of deionised water, respectively and administered at 0.1 ml per egg.

Source and management of fertile eggs

For each of the experiments, a total of 300 hatching eggs were procured from reputable hatcheries. The eggs were fumigated using potassium tetraoxomanganate VII ($KMnO_4$) and formalin at a ratio 1 : 2. The treatment lasted for 20 minutes in a closed chamber. The eggs were set in egg trays in a Chick Master® incubator with broad ends upward to prevent rupture of the air cell. Temperature (37.5–37.8 °C) and humidity (60–65 %) were automatically regulated. Egg turning was done manually on an hourly basis to prevent developing embryos from sticking to the shell, and also to ensure uniform distribution of nutrients.

Table 1. Experimental layout

Treatment groups	*In ovo* injection
Group I	Control (no *in ovo* supplementation)
Group II	*In ovo* supplementation with 80 µg.egg^{-1} of inorganic salts of zinc (Zn 351.80 µg.100 ml^{-1} deionised water)
Group III	*In ovo* supplementation with 16 µg.egg^{-1} of inorganic salt of copper (Cu 62.87 µg.100 ml^{-1} deionised water)
Group IV	*In ovo* supplementation with 80 µg.egg^{-1} of inorganic salts of zinc and 16 µg.egg^{-1} copper

A total of 300 hatching eggs (only 148 (59.20 %) and 232 (90.27 %), respectively, were fertile) each of Arbor Acre and Cobb 500 strains of broiler chickens were used in both experiments. Each of the *in ovo* treatment group (control, inorganic salts of zinc, copper and their combination) contained 37 and 58 fertile eggs in Experiment I and II, respectively. The treatment groups are shown in Table 1.

On the 18th day of incubation, the candled eggs (*in ovo* groups) were injected with nutrients (organic salts of zinc and copper) into amnion using a 24-gauge hypodermic needle. Before injections, the site was sterilised with 10 % ethanol and the injections were done at the broad end of the egg. Following *in ovo* feeding, the injection site was sealed with sterile paraffin and the eggs transferred to the hatching compartment. The *in ovo* injection of each treatment was completed within 30 minutes of taking the eggs out from the incubator.

Post-hatch chick and management

In Experiment I, a total of 21 chicks hatched (14.19 % hatchability); 10 chicks (27.02 % hatchability) from the control; 2 chicks (5.41 % hatchability) from Zn-injected eggs; 4 chicks (10.81 % hatchability) from Cu-injected eggs and 5 chicks (13.51 % hatchability) from ZnCu-injected eggs. The resulting chicks were distributed into replicate with unequal number of replications on the basis of *in ovo* treatment groups. This thereby informed the need for the second experiment but with a different strain (Cobb 500) of broiler chickens. In Experiment II, a total of 156 chicks hatched (67.245 % hatchability); 51 chicks (87.93 % hatchability) from the control; 53 chicks (91.38 % hatchability) from Zn-injected eggs; 34 chicks (58.625 % hatchability) from Cu-injected eggs, and 18 chicks (31.03 % hatchability) from ZnCu-injected eggs. The first two treatment groups were replicated equally (10 birds per replicate of 5), Cu-injected treatment group were replicated as 10 birds per replicate of 3 and ZnCu-injected treatment groups were replicated on 6 birds per replicate of 3.

The chicks were brooded for 2 weeks and fed commercial feeds containing 23 % crude protein, 3200 kcal. kg^{-1} metabolisable energy, 0.45 % available P, 190.30 ppm Zn and 29.30 ppm Cu at the starter phase, and 20 % crude protein, 3000 kcal.kg^{-1} metabolisable energy, 0.40 % available P, 88.10 ppm Zn, and 13.80 ppm Cu at the finisher phase. They were served fresh clean water *ad libitum*. They were raised intensively in a deep litter system for 7 weeks

in Experiment I and for 5 weeks (due to COVID-19 restriction on movement) in Experiment II with all necessary medications and vaccinations strictly adhered to.

Data collection

Hatching traits

a) Egg weight was determined by weighing the hatchling eggs on a sensitive weighing scale with the average weight recorded.
b) Percentage hatchability was calculated as: % Hatchability = (Number of hatched chicks/ Number of fertile eggs) × 100.
c) Weight (g) of the chicks at hatch was determined by weighing the chicks hatched in each replicate and the average weight was calculated.
d) Chicks to egg ratio was determined as the ratio of the chick weight to egg weight.

Gut morphometry

On the 7th and 49th day of post-hatch, two birds of average weight from each replicate were slaughtered by cervical dislocation for gut development studies. Gut morphometry was done by recording the weights of gizzard, proventriculus, liver as well as the weight and length of the duodenum, jejunum, ileum and caecum which were expressed as cm per 100 g live weight.

Post-hatch growth performance parameters

Feed intake

This was measured and recorded on replicate basis at the end of every week. Left over feed was subtracted from the amount of feed offered and the average feed intake was recorded as well. Feed wastage was greatly reduced by using a feeding trough with a lip and it was ensured that the trough was one-third filled at all times. Also, the feeding trough was hung at the level of the birds' back.

The following calculations were done on replicate basis:

Feed intake [g] = Feed offered [g] – Feed left [g]

Average feed intake [g.bird^{-1}] = (Feed intake/Number of birds)

Body weight

Body weight gain was calculated on replicate basis by subtracting the final weight from initial weight and average body weight gain was calculated.

Weight gain [g] = Final weight [g] – Initial weight [g]

Average weight gain [g.bird^{-1}] = (Weight gain/Number of birds)

Feed conversion ratio (FCR)

This is the proportion of feed converted into flesh by the birds. FCR was calculated as:

FCR = (Feed intake/Weight gain)

Cell-mediated immune response

At the 7th day of post-hatch, 1 bird per replicate was selected in experiment I because of the number of birds hatched while 2 birds per replicate were selected in experiment II, and their cell-mediated immune response to phytohaemaglutinin type P (PHA-P) was studied [7]. About 0.1 ml (concentration 1 mg.ml^{-1}) of PHA-P was injected at 3rd and 4th inter-digital space of the right foot. The left foot served as control and was injected with 0.1 ml phosphate-buffered saline (PBS). The foot web index was calculated as a difference between the swelling in the right and left feet before and after 24 hours of injection and expressed in millimetres [7].

The foot web/pad index was calculated as follows:

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

Where:

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

R1 = Thickness of the right foot web before injection

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

L1 = Thickness of the left foot web before injection

Humoral immune response

The antibody response to the Sheep Red Blood Cell (SRBC) was studied at the 21st day post-hatch, wherein 1 ml of 1 % SRBC was injected intravenous into the birds. The SRBC was washed thrice and centrifuged at 704 g for 10 minutes after each washing. After 5 days of SRBC immunization, 1 bird per replicate was selected in Experiment I (because of the number of birds hatched) and 2 birds per replicate in Experiment II, and 2 ml of blood was collected from the wing vein and the antibody titre was recorded by haemaglutination (HA) titre as enunciated in previous studies [31, 37].

Collection of blood samples

On the 7th day in both experiments and at 49th and 35th day post-hatch in experiment I and II, respectively, two birds per replicate were selected for blood collection; 2 ml of the blood samples was collected through the jugular vein into the blood sample bottles for the determination of serum biochemical indices using standard procedures [18]. All blood samples were analysed using routine laboratory procedures [12].

Determination of serum parameters

Serum total protein, albumin and cholesterol were determined spectro-photometrically according to the method [35] using Randox(R) diagonistic kit manual. Alanine aminotransferase was determined spectro-photometrically according to the method [28] using a commercial Randox kit.

Statistical analysis

The data collected were subjected to one-way Analysis of Variance in a Completely Randomized Design. Significant ($P < 0.05$) difference among means was separated using Tukey test as contained in the Minitab® version 17.1.0 [26].

RESULTS

Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on hatching traits of Arbor Acre and Cobb 500 strains of broiler chickens

Figure 1 shows the effects of *in ovo* injection of in organic salts of zinc, copper and their combination on hatching traits of Arbor Acre strain of broiler chickens. The highest hatchability of 27.02 % was obtained from the eggs of the control group, while the least hatchability of 5.41 %

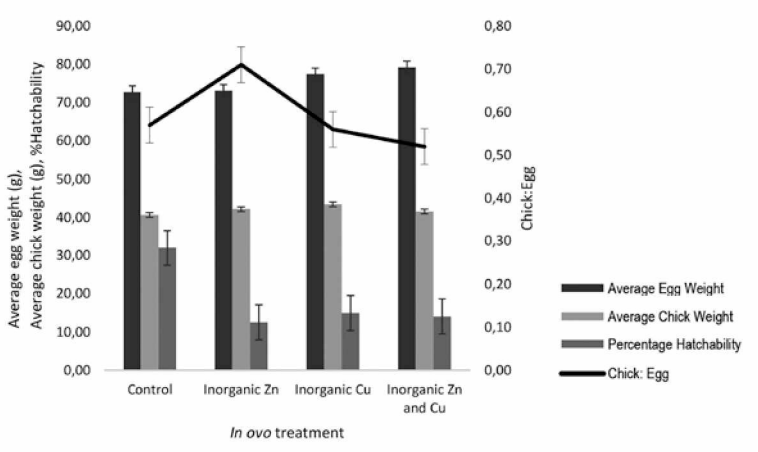

Fig. 1. Effect of *in ovo* injection of inorganic salts of Zn, Cu and their combination on hatching traits of Arbor Acre broiler chickens

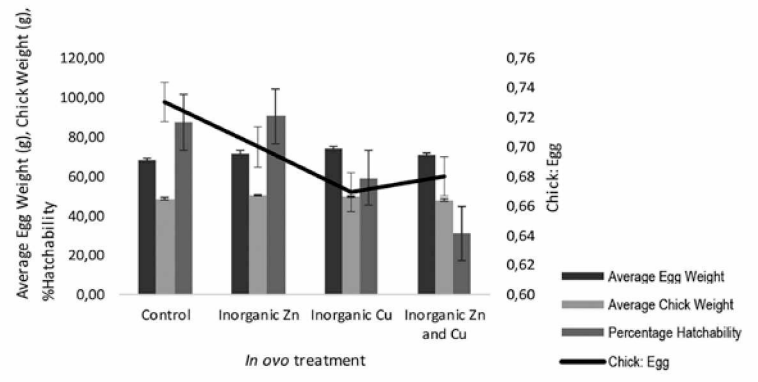

Fig. 2. Effect of *in ovo* injection of inorganic salts of Zn, Cu and their combination on hatching traits of Cobb 500 broiler chickens

was recorded in the eggs of the *in ovo* injection of inorganic salts of zinc. Hatchability of 13.51 % and 10.81 % were recorded in eggs of the *in ovo* injection of the combination of inorganic salts of zinc and copper (ZnCu) and eggs of the *in ovo* injection of salts of copper, respectively. It was observed that the highest chick weight of 41.58 g was obtained in chicks from eggs of the *in ovo* ZnCu; this was followed by chicks resulting from eggs injected with inorganic copper salts (34.77 g) and those in the control group (25.60 g). The lowest chick weight of 16.88 g was obtained in chicks from the *in ovo* injection of inorganic Zn salts. Chick to egg ratio ranged from 0.36 to 0.52, with the least values recorded in groups of the *in ovo* injection of inorganic salt of zinc while the highest chick to egg ratio was obtained in the eggs of the *in ovo* treatment injected with the combination of inorganic salts of zinc and copper.

In Figure 2, the effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on hatching traits of Cobb 500 strains of broiler chickens are shown. Highest hatchability of 91.38 % was obtained in eggs from the *in ovo* injection of inorganic salt of zinc while the least hatchability of 31.03 % was recorded in eggs from the *in ovo* injection of salts of zinc and copper combined. Eggs injected with copper salt and eggs in the control, both had hatchability 58.62 % and 87.93 %, respectively. The highest chick weight of 50.52 g was obtained in birds from eggs with the *in ovo* injection of zinc salt; this was followed by birds resulting from eggs injected with inorganic copper salt (49.87 g) and those in the control group (49.33 g). The lowest chick weight of 49.06 g was obtained in birds from the *in ovo* injection of the combination of inorganic salt of zinc and copper. Chick to egg ratio ranged from 0.67 to 0.73, with least values recorded in birds from the *in ovo* injection of inorganic copper while the highest chick to egg ratio was obtained in eggs from the control group.

Effects of *in ovo* injection of inorganic salts of copper, zinc and their combination on growth performance of Arbor Acre and Cobb 500 strains of broiler chickens

Table 2 shows the effects *in ovo* injection of inorganic salts of copper, zinc and their combination on growth performance of Arbor Acre strain of broiler chickens. The effects of *in ovo* injection of inorganic salts of copper, zinc and their combination (ZnCu) showed significant ($P < 0.05$) effect on the daily feed intake of Arbor Acre strain of broiler chickens. Birds from eggs injected with in-

Table 2. Effects of *in ovo* injection of inorganic salts of copper and zinc on growth performance of Arbor Acre and Cobb 500 strains of broiler chickens

Parameters	*In ovo* injection					
	Control	Zn	Cu	ZnCu	SEM	P-value
Arbor Acre						
Initial weight [g.bird^{-1}]	44.67	ND	44.50	47.13	2.18	0.011
Final weight [g.bird^{-1}]	1440.00	ND	2100.00	1683.00	124.0	0.140
Daily weight gain [g.bird^{-1}.d^{-1}]	28.48	ND	41.95	33.39	2.53	0.140
Daily feed intake [g.bird^{-1}.d^{-1}]	76.78^{b}	ND	129.96^{a}	86.51$^{a, b}$	4.88	0.036
Feed conversion ratio	**2.71**	**ND**	**3.10**	**2.59**	**0.18**	**0.366**
Cobb 500						
Initial weight [g.bird^{-1}]	65.56	67.76	61.90	65.28	1.59	0.152
Final weight [g.bird^{-1}]	928.90	889.10	876.50	904.20	85.30	0.974
Daily weight gain [g.bird^{-1}.d^{-1}]	24.67	23.47	23.27	23.97	2.46	0.977
Daily feed intake [g.bird^{-1}.d^{-1}]	47.85^{b}	51.83a^{b}	58.56^{a}	52.90$^{a, b}$	1.88	0.020
Feed conversion ratio	**1.95**	**2.23**	**2.67**	**2.24**	**0.27**	**0.365**

$^{a, b}$ – Means on the same row having different superscripts differ significantly ($P < 0.05$); ZnCu – combination of zinc and copper; ND – No data (only 2 chicks hatched, hence no replication); SEM – Standard error of mean; P-value – Probability value

Table 3. Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphometry of Arbor Acre and Cobb 500 strains of broiler chickens at day 7

Parameters	*In ovo* injection					
	Control	Zn	Cu	ZnCu	SEM	P-value
Arbor Acre						
Live weight [g.bird^{-1}]	**83.50**	**ND**	**97.00**	**59.00**	**10.93**	**0.199**
Organs [%]						
Heart	0.83	ND	0.76	1.17	0.195	0.503
Liver	3.55	ND	2.42	4.72	1.16	0.527
Proventriculus	1.05	ND	0.73	0.58	0.95	0.575
Gizzard	7.26	ND	5.01	9.96	2.61	0.542
Gut						
Duodenum [%]	2.41	ND	1.76	2.270	0.32	0.532
Duodenum [cm.100 g^{-1} LW]	13.57	ND	9.90	22.14	4.47	0.332
Jejunum [%]	3.13	ND	1.56	3.12	0.56	0.335
Jejunum [cm.100 g^{-1} LW]	28.69	ND	22.27	40.98	5.63	0.261
Ileum [%]	4.01	ND	1.28	3.04	0.55	0.161
Ileum [cm.100 g^{-1} LW]	30.43	ND	22.99	43.98	4.13	1.333
Caecum [%]	1.30	ND	0.64	1.55	0.41	0.482
Caecum [cm.100 g^{-1} LW]	14.78	ND	9.18	18.28	1.57	0.121
Colon [%]	0.86	ND	0.31	1.01	0.15	0.175
Colon [cm.100 g^{-1} LW]	4.77	ND	3.71	6.12	1.33	0.484
Cobb 500						
Live weight [g.bird^{-1}]	**141.50**	**148.50**	**156.00**	**115.50**	**12.50**	**0.258**
Organs [%]						
Heart	1.00	0.97	0.99	0.91	0.09	0.905
Liver	4.52	4.94	4.54	5.00	0.72	0.937
Proventriculus	0.82	1.03	0.81	1.00	0.09	0.309
Gizzard	6.94	6.89	7.67	8.43	0.74	0.493
Gut						
Duodenum [%]	1.73	1.79	1.94	2.05	0.08	0.134
Duodenum [cm.100 g^{-1} LW]	11.49	10.46	9.66	12.06	0.96	0.404
Jejunum [%]	3.09	2.80	2.93	2.30	0.41	0.962
Jejunum [cm.100 g^{-1} LW]	22.83	24.94	17.68	25.77	2.99	0.348
Ileum [%]	2.68	2.660	2.21	3.16	0.42	0.533
Ileum [cm.100 g^{-1} LW]	22.85	25.73	19.31	25.74	2.24	0.278
Caecum [%]	0.818	0.68	0.61	0.88	0.14	0.546
Caecum [cm.100 g^{-1} LW]	10.74	10.10	8.32	10.63	1.83	0.776
Colon [%]	0.48	0.34	0.48	0.43	0.06	0.333
Colon [cm.100 g^{-1} LW]	2.984	2.70	2.75	2.86	0.28	0.819

ZnCu – combination of zinc and copper; LW – Live weight; SEM – Standard error of mean;
ND – No data (only 2 chicks hatched hence no replication); P-value – Probability value

Table 4. Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphometry of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively

Parameters	*In ovo* injection					
	Control	Zn	Cu	ZnCu	SEM	P-value
Arbor Acre						
Live weight [g.bird^{-1}]	**2063.00**	**ND**	**2100.00**	**1765.00**	**274.66**	**0.573**
Organs [%]						
Heart	0.59	ND	0.62	0.53	0.17	0.910
Liver	1.92	ND	1.91	2.06	0.20	0.795
Proventriculus	0.39	ND	0.43	0.38	0.06	0.884
Spleen	0.13	ND	0.14	0.17	0.05	0.742
Gizzard	1.82	ND	2.38	2.03	0.16	0.191
Pancreas	0.23	ND	0.24	0.29	0.06	0.526
Gut						
Duodenum [%]	0.74	ND	0.38	0.82	0.09	0.704
Duodenum [cm.100 g^{-1} LW]	1.57	ND	0.76	1.73	0.25	0.153
Jejunum [%]	1.32	ND	1.00	1.34	0.17	0.521
Jejunum [cm.100 g^{-1} LW]	3.15	ND	2.88	3.49	0.53	0.728
Ileum [%]	1.08	ND	1.05	1.17	0.22	0.921
Ileum [cm.100 g^{-1} LW]	3.29	ND	2.60	3.55	0.71	0.735
Caecum [%]	0.49	ND	0.52	0.71	0.15	0.420
Caecum [cm.100 g^{-1} LW]	1.94	ND	1.71	2.11	0.41	0.829
Colon [%]	0.15	ND	0.16	0.19	0.06	0.852
Colon [cm.100 g^{-1} LW]	0.37	ND	0.39	0.47	0.09	0.634
Cobb 500						
Live weight [g.bird^{-1}]	**1366.70**	**1333.30**	**1033.30**	**1316.70**	**84.60**	**0.077**
Organ [%]						
Heart	0.41	0.48	0.62	0.51	0.05	0.154
Liver	2.70	2.59	2.91	2.77	0.47	0.969
Proventriculus	0.42	0.62	0.07	0.59	0.07	0.127
Spleen	0.11	0.11	0.14	0.13	0.02	0.622
Gizzard	3.75	3.54	3.764	3.69	0.44	0.982
Pancreas	0.25	0.19	0.23	0.29	0.04	0.504
Gut						
Duodenum [%]	0.88	1.02	1.08	9.57	0.13	0.756
Duodenum length [cm.100 g^{-1} LW]	2.145	2.19	2.83	2.38	0.23	0.217
Jejunum [%]	2.73	8.02	2.89	2.13	0.39	0.445
Jejunum [cm.100 g^{-1} LW]	5.69	6.65	7.01	6.09	0.74	0.618
Ileum [%]	2.29	2.57	2.72	2.04	0.29	0.402
Ileum [cm.100 g^{-1} LW]	6.12	7.12	7.07	6.57	0.65	0.680
Caecum [%]	0.76	0.70	0.90	0.88	0.07	0.225
Caecum [cm.100 g^{-1} LW]	2.00	3.00	4.00	3.00	0.23	0.013
Colon [%]	0.53	0.29	0.36	0.43	0.10	0.411
Colon [cm.100 g^{-1} LW]	0.60	0.63	0.78	0.80	0.1	0.413

ZnCu – combination of zinc and copper; LW – Live weight; SEM – Standard error of mean;
ND – No data (only 2 chicks hatched hence no replication); P-value – Probability value

organic copper salt and birds from eggs injected with combined salts of ZnCu were statistically similar and followed by birds resulting from eggs of the control group (76.78 g). The same trend was observed in the Cobb 500 strain of broiler chickens. Birds from eggs injected with copper had the highest ($P<0.05$) daily feed intake (58.56 g) followed by birds from eggs injected with ZnCu combination, zinc and the un-injected eggs.

Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphology of Arbor Acre and Cobb 500 strains of broiler chickens at day 7

In Table 3, the effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphometry of Arbor Acre and Cobb 500 strains of broiler chickens at day 7 of age are shown. In both strains of broiler chickens, there were no significant ($P>0.05$) variations in all the organ and gut morphometry parameters measured.

Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphology of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively

The effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on organ development and gut morphometry of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively are shown in Table 4. In both strains of broiler chickens, there were no significant ($P>0.05$) differences in all the parameters measured.

Effects of *in ovo* injection of inorganic zinc, copper and their combination on cell-mediated immunity and humoral immunity of Arbor Acre and Cobb 500 strains of broiler chickens

The effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on cell-mediated immunity and humoral immunity (after 24 hours of injecting phytohaemagglutinin type-P (PHA-P) of Arbor Acre and Cobb 500 strains of broiler chickens are shown in Figures 3 and 4, respectively. In Arbor Acre (Figure 3), the highest cell-mediated immune response was recorded in birds on the control group and this reduced across the treatment groups. The same trend was observed in the humoral immunity but with upsurge in the humoral immunity of the birds using *in ovo* injection of the combination of inorganic salts of zinc and copper. In Cobb 500 strain (Figure 4), birds from eggs with *in ovo* injection of inorganic salt of

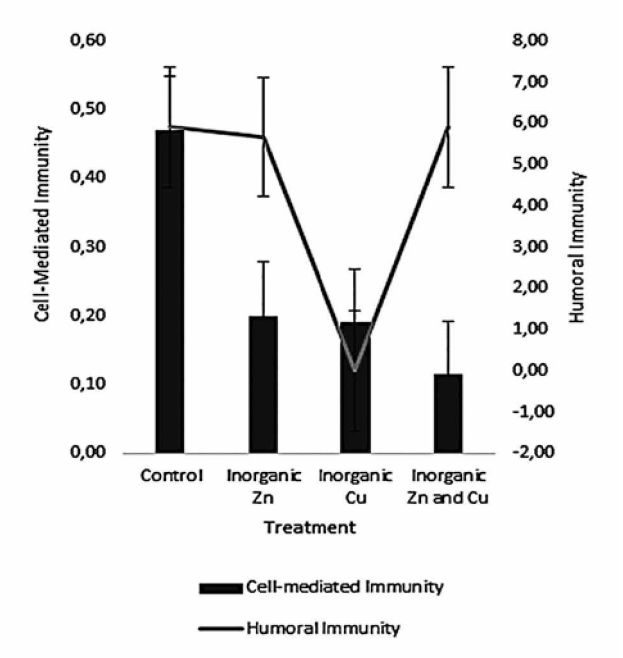

Fig. 3. Effect of *in ovo* injection of inorganic salts of Zn, Cu and their combination on cell-mediated immunity and humoral immunity of Arbor Acre broiler chickens

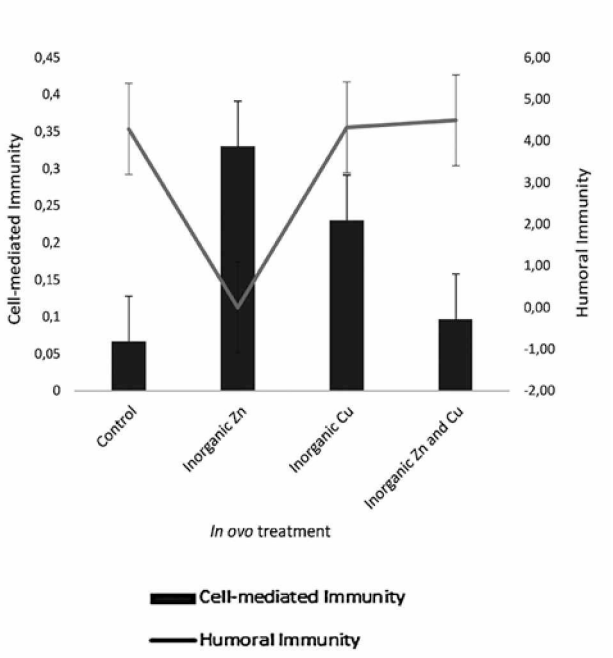

Fig. 4. Effect of *in ovo* injection of inorganic salts of Zn, Cu and their combination on cell-mediated immunity and humoral immunity of Cobb 500 broiler chickens

Table 5. Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on serum biochemical indices of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively

Parameters	*In ovo* injection					
	Control	Zn	Cu	ZnCu	SEM	P-value
Arbor Acre						
Total protein [g.dl^{-1}]	2.60^{b}	ND	4.60^{a}	4.00^{a}	0.11	0.012
Albumin [g.dl^{-1}]	1.50^{b}	ND	2.60^{a}	2.35$^{a, b}$	0.12	0.040
Globulin [g.dl^{-1}]	1.10	ND	2.00	1.65	0.22	0.191
Cholesterol [g.dl^{-1}]	76.10	ND	71.50	56.00	6.96	0.259
Triglyceride [g.dl^{-1}]	99.70	ND	127.90	118.1	21.63	0.698
Low density lipoprotein [g.dl^{-1}]	19.65	ND	15.60	9.40	3.61	0.274
Very low-density lipoprotein [g.dl^{-1}]	19.95	ND	25.60	23.65	4.34	0.698
High density lipoprotein [g.dl^{-1}]	36.55	ND	30.30	22.95	7.43	0.479
Aspartate aminotransferase [U.l^{-1}]	61.00	ND	80.00	60.50	4.71	0.188
Alanine aminotransferase [U.l^{-1}]	14.00	ND	14.00	16.50	3.71	0.850
Cobb 500						
Total protein [g.dl^{-1}]	4.50	4.90	5.45	5.40	0.33	0.282
Albumin [g.dl^{-1}]	2.70	3.15	3.10	3.25	0.12	0.098
Globulin [g.dl^{-1}]	1.85	1.75	2.35	2.15	0.43	0.759
Cholesterol [g.dl^{-1}]	61.2	88.9	78.6	102.5	17.0	0.461
Glucose [g.dl^{-1}]	170.1	144.0	116.9	110.7	17.4	0.203
Triglyceride [g.dl^{-1}]	74.05	70.40	69.40	72.05	2.87	0.701
Low density lipoprotein [g.dl^{-1}]	16.45	25.90	20.65	29.35	6.04	0.520
Very low-density lipoprotein [g.dl^{-1}]	14.80	14.10	13.90	14.00	0.58	0.727
High density lipoprotein [g.dl^{-1}]	29.90	48.80	44.00	58.70	11.6	0.454
Aspartate aminotransferase [U.l^{-1}]	82.00	81.50	79.50	77.50	5.76	0.939
Alanine aminotransferase [U.l^{-1}]	28.50	30.50	26.50	28.00	2.17	0.657

$^{a, b}$ – Means on the same row having different superscripts differ significantly ($P < 0.05$); ZnCu – combination of zinc and copper; ND – No data; SEM – Standard error of mean; P-value – Probability value

zinc, had the highest cellular immunity of 0.33 mm after 24 hours of injection; this was followed by birds from eggs on *in ovo* injection of inorganic salts of copper (0.23 mm) and birds from eggs on *in ovo* injection of inorganic salts of zinc and copper with 0.09 mm, respectively. The lowest cellular immunity of 0.06 mm was obtained in the birds from eggs in the control group after 24 hours of injecting PHA-P. Humoral response to sheep red blood cell (SRBC) ranged from 4.49 mm to 4.28 mm. The lowest value was recorded in birds from eggs using *in ovo* injection of inorganic salts of zinc while the highest value was recorded in birds injected with the combination of inorganic salts of zinc and copper.

Effects of *in ovo* injection of inorganic salts of zinc, copper and their combination on serum biochemical indices of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively

Table 5 shows the effect of *in ovo* administration of inorganic salts of zinc, copper and their combination on

serum biochemical indices of Arbor Acre and Cobb 500 strains of broiler chickens at days 49 and 35, respectively. Chicks from eggs injected with Cu had the highest ($P<0.05$) total protein production, followed by chicks resulting from ZnCu injected group while the least total protein value was recorded in chicks resulting from the control group. The same trend was also observed in serum albumin. In Cobb 500 strain of broiler chickens, there were no significant ($P>0.05$) differences in all the parameters measured.

DISCUSSION

The least hatchability results obtained from eggs of the *in ovo* injection of inorganic salt of zinc treatment group in Experiment I (Arbor Acre strain) contradicted the result of [32] on improved hatchability in the Zn-injected hatchings eggs. The difference may be attributed to different strains of broiler chickens used. In addition, there were many other factors that could contribute to the varying hatchability of *in ovo* treatment groups including: the source of the eggs, the age of the breeder flock, nutrition, the egg storage condition before setting, and the injection time. The highest percentage of hatchability obtained in the control group on the *in ovo* injection group was in line with the findings [2] that *in ovo* supplementation of trace minerals enriched solution did not show any significant difference in hatchability of *in ovo* injected group compared to eggs without *in ovo* supplementation. In Experiment II, the hatchability result obtained in Zn-injected eggs of Cobb 500 strain of broiler chickens correlate with the results that affirmed [32] that zinc as a crucial trace mineral for embryonic development of broiler chickens. Zinc is distributed throughout the body and plays a critical role in improving: reproduction, development of blood cells, immune system function, and bone development [1].

In Experiments I and II, *in ovo* injection of these minerals (Zn, Cu and ZnCu) did not significantly affect chick weight and this corroborated the findings [19] that *in ovo* injection particularly of nano-zinc had no influence on hatch weight. There have been varying results on growth traits with *in ovo* administration of trace minerals in broiler chickens where no effect [30] and increased body weights with *in ovo* administered groups over control group were reported [32]. These varying results can be attributable to the differences in the strains of broiler chickens and the growth-promoting effects of various substances administered *in ovo*. Similarly, birds from Cu-injected eggs recorded the highest feed intake in both Experiments I and II of this study.

These results have demonstrated the positive effect of *in ovo* injection of inorganic salt of Cu especially at minute quantities on feed intake stimulation in broiler chicken. According to one study [38], improved feed intake was observed in birds fed dietary copper at the level of 200 ppm when compared with birds fed dietary copper at 600 and 800 ppm. The authors attributed the improved intake at 200 ppm to the reduction of pathogenic microorganisms and improved activity of digestive enzymes such as protease, amylase and lipase, which ensured better digestion and utilization of the feed within the digestive tract.

The non-significant differences in digestive organs length observed in both Experiments I and II at different ages were not in line with the findings [36]. The authors indicated that *in ovo* feeding improved gastrointestinal tract development of hatchlings and functionally similar to that of conventional 2-day old chicks offered feed immediately after hatch. In addition, it was reported [14] that the development of gut occurs throughout incubation and when the embryo starts consuming amniotic fluid orally, intestinal weight increases from approximately 1 % at 17 days of incubation to 3.5 % at hatch.

The literature is limiting on the influence of *in ovo* feeding of inorganic salts on serum biochemical indices of broiler chickens. However, at day 49 of Experiment I on Arbor Acre strain of broiler chickens, the highest total protein and albumin were observed in birds from eggs with *in ovo* injection of Cu and the values were comparable to the values obtained in birds from ZnCu injected eggs. This indicated that albumin is synthesized in the right quantity for transporting insoluble substance in the blood and aids the maintenance of oncotic pressure [10]. A higher concentration denotes dehydration while lower concentration may be due to factors such as malnutrition and infection [8]. Both total protein and albumin values were within the normal range (TP 3.0–4.9 $mg.dl^{-1}$ and 1.17–2.74 $g.dl^{-1}$, respectively) reported [25] for healthy broiler chickens.

In Experiment II, improved cellular immunity was obtained in birds from eggs of the *in ovo* injection of Zn and this same trend was seen in birds on other *in ovo* treatment groups. Also, the highest humoral immunity was obtained

in birds from eggs injected with the combination of zinc and copper salt. This result was at variance with findings [32] of non-significance in the growth of immune organs and response to PHA-P or SRBC thereby suggesting that *in ovo* injection of inorganic salts of Zn, Cu and their combination might not be immunomodulatory in broiler chickens of improved genetic lines. However, the results obtained in this study is in line with the reports on *in ovo* injection of lysine [24], arginine [21] and 8 $\mu g.egg^{-1}$ of inorganic Cu ($CuSO_4$), which were found to enhance the immune response of broiler chickens [14].

CONCLUSIONS

From this study, it could be concluded that:

- administration of inorganic salts Zn and Cu *in ovo* reduced percentage hatchability in Arbor Acre strain but improved percentage hatchability in Cobb 500 strain of broiler chickens;
- *in ovo* administration of the combination of inorganic salts of Zn and Cu did not significantly affect the chick weights of both strains of broiler chickens but there was a numerical increase in the final weights of the birds due to *in ovo* administration of inorganic salt of Cu in Arbor Acre broiler chicken from significantly increased daily feed intake;
- *in ovo* injection of inorganic salts of Zn and Cu did not significantly affect the gut development of both strains of broiler chickens;
- administration of inorganic salts of Zn and Cu *in ovo* had an appreciable significant influence on the cellular and humoral immunity of Cobb 500 strain of broiler chicken, thereby, indicating that the ability of the inorganic nutrients to enhance antibody production;
- the total protein and albumin of the Arbor Acre strain of broiler chickens were significantly increased by the *in ovo* administration of inorganic salts of Zn, Cu and their combination.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in the conception, design and implementation of the study.

ACKNOWLEDGEMENT

The authors wish to gratefully acknowledge the grants-in-aid received from the Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, for the research.

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Received August 12, 2022
Accepted November 23, 2022