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HATCHING TRAITS AND POST-HATCH PERFORMANCE OF BROILER CHICKENS ON *IN OVO* FEEDING OF AQUEOUS EXTRACTS OF GARLIC, MUSHROOM AND THEIR COMBINATION

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ABSTRACT

Feeding embryos with exogenous feed components prior to hatching has been reported to positively improved the growth and immune response in poultry. This study investigated the effects of *in ovo* administration of aqueous extracts of garlic, oyster mushroom and their combination on hatching traits and post-hatch performance of broiler chickens. A total of 230 broiler hatching eggs were sorted and incubated. On candling, 200 (85.11 % fertility) fertile eggs were obtained and distributed into five treatments: control, *in ovo* injection of garlic, oyster mushroom, combination of garlic and oyster mushroom, and de-ionized water (SHAM) at 0.1 ml.egg^{-1} in each treatment. *In ovo* injection was done on 18th day of incubation. Data collected were subjected to One-Way Analysis of Variance. Chick weight was the highest (P < 0.05) in birds hatched from eggs injected with aqueous extracts of garlic (50.91 g per chick). The highest proportions (P < 0.05) of heart (0.65 %) and spleen (0.18 %) were observed in birds from eggs injected with oyster mushroom and the combination of garlic and oyster mushroom, respectively. Significantly highest lymphocyte counts (P < 0.05) were found in birds from eggs injected with aqueous extracts of garlic, and the combination of garlic and oyster mushroom. The best (P < 0.05) feed conversion rate (FCR) (1.82) was recorded in birds from eggs on *in ovo* injection of aqueous extract of oyster mushroom. The study suggests that *in ovo* injection of aqueous extracts of garlic and oyster mushroom at 0.1 ml per egg can enhance chick weight, heart, spleen functions and overall growth performance in broiler chickens.

Key words: blood profile; broiler chickens; growth performance; hatching traits; *in ovo*; phytogenic

INTRODUCTION

A prominent early nutrition technique that could provide further opportunities to influence the development of an embryo and overcome the constraints of nutrient limitation during late incubation phase is *in ovo* feeding. This technique

provides the opportunity to supply essential nutrients, nutraceuticals, and functional foods to improve growth and development of embryo. Feeding the embryo before hatch by *in ovo* administration of nutrients components was reported to positively affect hatchability, development of the digestive tract, body weight and nutritional status of the hatchlings and immune system [20, 32]. The authors [33] reported that due to rapidly growing embryos and the swift metabolic turnover of modern broiler chicks, some essential nutrients become depleted during embryogenesis thereby suggestive of the relevance of *in ovo* feeding in poultry production.

Poultry production has gained much popularity in a bid to produce affordable and safe protein for the growing the populace over the years. This encouraged producers to adopt intensive rearing as it offers more control on the management of poultry and the use of antibiotics which has led to improved feed efficiency, increased growth rates and reduced food production costs. Unfortunately, this intensive system led to the development of several challenges such as overcrowding, rapid disease spread, lower immunity, economic losses and antibiotic resistance leading to a possible reduction in effectiveness of treatment options for both animals and humans [24, 31].

To mitigate the issue of antibiotic residues in poultry products, the farmers are seeking some alternatives. This has led to studies [9, 24] on the use of phytobiotics, including spices and herbs, as substitutes with prophylactic and therapeutic effects as those of synthetic antibiotics but with lesser side effects. These alternatives, such as probiotics, prebiotics, synbiotics, acidifiers and phytobiotics [2], are natural, non-toxic, and leave no residues in poultry products. Phytobiotics, including turmeric, ginger, garlic, mushrooms and their extracts, stimulate appetite and endogenous secretions such as enzymes that are antimicrobial, coccidiostatic or anthelminthic [16, 21].

Garlic has been reported to possess useful pharmacological substances [21, 23]. Freshly crushed garlic contains allicin, alliin, ajoene, di-allylsulphide, dithiin, S-allyl-cysteine. Garlic as a natural feed additive in poultry nutrition is of great benefit for broiler growers due to its antibacterial, anti-inflammatory, antiparasitic and immunomodulatory properties [22]. It is one of the highly investigated medicinal plants used as a growth promoter in broiler chickens [3, 15].

Mushrooms are fruiting bodies of fungi that are known to offer several benefits such as rapid growth, improved health, and increased resistance to and protection from pathogens [11]. Moreover, mushrooms are a rich source of natural antibiotics. The cell surfaces of mycelia secrete antibiotics, known as exudates, most of which are secondary metabolites [35]. Some of these antibiotics and enzymes target specific microbes. Mushrooms are also rich in B vitamins (B2, B3, folate, B5) and vitamin D, as well as in minerals such as P, Se, Cu and K that are required by poultry. This implies that mushrooms could be utilized as a feed supplement to improve growth performance and health of poultry [30] as well as reproductive performance.

The studies [8, 12] on the use of garlic, oyster mushroom and other phyto-additives in diets of birds have produced consistent results. However, there is limited information on the *in ovo* injection of garlic and oyster mushroom extracts and their effects on hatching traits and post-hatch performance of broiler chickens.

MATERIALS AND METHODS

Ethical consideration

This study was carried out according to the Animal Use and Care Committee guidelines of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, and that of Federal Republic of Nigeria (A17 LFN, 2004). Efforts were made to lessen the pain and harm to experimental birds.

Experimental site

The hatchery aspect of the experiment was carried out at the Hatchery, College of Animal Science and Livestock Production while the field trial was undertaken at the Poultry Unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Nigeria, located within latitude 7^0 15' 59.66'' N, longitude 3^0 26' 13.64''.

Source of fertile eggs

A total of 250 hatching eggs of Cobb500 broiler chickens were purchased from a reputable breeder farm at Ibadan, Oyo State, Nigeria.

Preparation of aqueous extracts of garlic and oyster mushroom

Garlic and oyster mushroom (200 g each) were purchased from the open market, washed to remove dirt and

blended separately into a paste using a blender at 448 x g. They were added to water at a ratio of 1:2 each (200 g of garlic and oyster mushroom to 400 ml of water, respectively) and mixed thoroughly. Then the mixture was heated for 15 minutes to a boiling point using a gas burner, and allowed to cool to room temperature. Each mixture was then screened using a cheese cloth and 20 % extract was prepared using a de-ionised water as reported by Sogunle et al. [28].

Preparation of the hatchery

The hatchery equipment (incubator, setting trays and hatching crates) including its surroundings were cleaned and disinfected to ensure that they are free disease agents. The incubator was further fumigated with formaldehyde and potassium permanganate ($KMnO_4$) in the ratio of 2:1.

Setting and management of hatching eggs during incubation

Hatching eggs were sorted to give 235 settable eggs. The hatching eggs were weighed and fumigated with formaldehyde and potassium permanganate in the ratio of 2:1 in an enclosed chamber for 30 minutes. The eggs were set in egg trays in a chick master incubator with broad ends upward to prevent rupture of the air cell and incubated at 37.5–37.8 °C and relative humidity of 56 to 60 %. They were turned automatically on hourly basis to prevent the developing embryos from sticking to the shell and also ensure uniform distribution of nutrients during 18 days in the setting compartment.

On day 14 of incubation, the eggs were candled with the use of an electrical hand-held device to detect the presence of developing embryos. The infertile eggs were discarded and the remaining 200 fertile eggs (85.11% fertility) were evenly assigned to five treatments, 40 eggs in each, using a completely randomized design: 1. Without *in ovo* injection (negative control); 2. *In ovo* injection of 0.1 ml of aqueous extracts of garlic; 3. *In ovo* injection of 0.1 m of aqueous extracts of oyster mushroom; 4. *In ovo* injection of 0.1 ml of aqueous extracts of the combination of garlic and mushroom at a ratio of 1:1 [29]; 5. *In ovo* injection of 0.1 ml of de-ionized water (positive control) sham.

The eggs were removed from the setter on the 18th day, the broad end of each egg was disinfected with 70 % ethanol to prevent pathogens and germs from getting access into the egg before a hole for injection was made. The injection into the amniotic fluid [27] was done by means of a 25 mm long [4], 24-gauge hypodermic needle, to deliver 0.1 ml of the aqueous extracts of garlic, mushroom or their combination, respectively. The *in ovo* injection for each of the treatments was completed within 10 minutes of taking the eggs out of the incubator. After the injection, the punctured hole was sealed with candle wax.

Post-hatch management of hatched chicks

After hatching, the hatched chicks (163 chicks in total, i.e. 81.5 % hatchability) from the treatments were reared using a deep litter housing system. The hatched chicks included: Treatment 1 (control) – 35 chicks (87.50 % hatchability); Treatment 2 (garlic) – 34 chicks (85 % hatchability); Treatment 3 (oyster mushroom) – 30 chicks (75 % hatchability); Treatment 4 (garlic and oyster mushroom) – 34 chicks (85 % hatchability); treatment 5 (sham) – 30 chicks (75 % hatchability).

The hatched chicks were brooded on the treatment basis of three replicates per treatment. Dry wood shavings were used as bedding and standard broiler feed and water were provided *ad libitum*. The diets, commercially prepared were phased and composed of: Pre-starter from 0–7 days: 2995.50 ME [kcal.kg^{-1}] and 22.47 % CP; Starter from weeks 1 to 3: 2994.10 ME [kcal.kg^{-1}] and 21.97 % CP; Finisher from weeks 3 to 5: 3050.65 ME [kcal.kg^{-1}] and 21.27 % CP.

Trace minerals 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. D_3, 1200 ICU; B-Complex 0.02 %, choline 0.05 % and salt 0.3 %. The study lasted 35 days.

Data collection

Hatching traits

The egg weight was measured before setting the eggs. The hatchability [%] was calculated using the formula:

$$\text{Hatchability [\%]} = \frac{\text{No. of hatched chicks per replicate}}{\text{No. of fertile eggs per replicate}} \times 100$$

Chick weight was measured at hatch and the average of each replicate was calculated. The chicks to eggs ratio was calculated. Dead in germ and dead in shell were determined on days 7 and 16 of incubation, respectively. They were calculated on replicate basis using the following formulae:

$$\text{Dead in germ [\%]} = \frac{\text{No. of dead in germ per replicate}}{\text{No. of fertile eggs per replicate}} \times 100$$

$$\text{Dead in shell [\%]} = \frac{\text{No. of dead in shell per replicate}}{\text{No. of fertile eggs per replicate}} \times 100$$

Gut morphometry

On 7th and 35th day 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 the gizzard, proventriculus and liver as well as the weight and length of the duodenum, jejunum, ileum and caecum which was expressed in cm.100 g^{-1} live weight.

Growth performance parameters

The growth performance indices including feed intake, average feed intake, weight gain, average weight gain, feed conversion ratio and mortality were calculated using the following formulae:

Feed intake [g] = Offered feed (g) - Leftover feed (g)

$$\text{Average feed intake [g.bird}^{-1}\text{]} = \frac{\text{Feed intake per replicate}}{\text{No. of birds per replicate}}$$

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

$$\text{Average weight gain [g]} = \frac{\text{Weight gain (g)}}{\text{No. of birds per replicate}}$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Cellular immune response

At 14th day post-hatch, 2 birds per replicate were selected and their cell-mediated immune response to phytohaemagglutinin type P (PHA-P) was studied using a standard method [6]. 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 it 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 48 hours of injection and expressed in millimetres using a micrometre screw gauge.

The foot web or pad index was calculated as follows:

Cell-mediated immune response

Where:

R2 = Thickness of the right foot web after 48 hours of injection

R1 = Thickness of the right foot web before injection

L2 = Thickness of left foot web after 48 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) were studied at 21st day post-hatch as follows: 5 ml of SRBC was added to 5 ml of Alsevier's solution and centrifuged at 700 x g for 10 minutes. The serum was decanted, PBS was added to make up 10 ml and centrifuged at 700 x g for 3 minutes. The process was repeated thrice after which 0.5 ml of the SRBC was added to 49.5 ml of PBS and refrigerated prior to use. The SRBC was washed to reduce the concentration, 0.1 ml of the treated SRBC was injected intravenously into the wing vein of 2 selected and tagged birds of average weight per replicate. After 5 days of SRBC immunization, the same 2 birds per replicate injected with treated SRBC were selected and 2 ml of blood was collected from the wing vein and the antibody titre was measured by haemagglutination (HA) titre [34].

Determination of blood profile

Blood was collected on day 35 of age, two birds per replicate were selected and bled through brachial vein into two test tubes, one containing ethylene diamine tetra acetic acid (EDTA) for haematological study and the other sterile test tube without EDTA. The second set of tubes was covered and centrifuged, serum separated out, decanted and deep-frozen for serum biochemical analyses. The haematological parameters measured were analysed according to standard procedures [26]. Packed cell volume (PCV) was determined using microhaematocrit capillaries. Haemoglobin concentration (Hb) was determined using cyanmethaemoglobin method which involves mixing five ml of Drabkin's solution (1000 ml of de-ionised water with 400 mg potassium ferricyanide, 280 mg potassium dihydrogen phosphate, 100 mg potassium cyanide and one ml of non-ionic detergent) with 20 µl of blood sample. The mixture was read using a colorimeter at 540 nm (green filter). Blood counts were determined by means of an improved Neubauer's chamber (area of 9 sq.mm^{-1} and depth of 0.1 mm). Serum biochemical parameters (total protein, albu-

min, globulin, creatinine, cholesterol, triglycerides) were analysed using commercially available test kits (Randox laboratory, United Kingdom, Model BT294QY).

Statistical analysis

The data collected were subjected to Completely Randomized Design [19]. Significantly ($P < 0.05$) different means among variables were separated using Tukey test as contained in the same statistical package. The statistical model of the experiment is as follows:

$Yij = \mu + Ti + \varepsilon ij$

Where: Yij is the individual observation ij,
μ is the overall mean effect,
Ti is the treatment effect i,
εij is the experimental error effect.

RESULTS

Effects of *in ovo* injection on hatching traits of broiler chickens

Table 1 shows the hatching traits of eggs after *in ovo* injection of aqueous extracts of garlic, oyster mushroom and their combination. The results showed that *in ovo* injection of garlic extract significantly ($P < 0.05$) influenced chick weight. Chicks hatched from eggs injected with 0. 1 ml of garlic showed the highest chick weight (50.91 g). The lowest chick weight of 44.38 g was recorded in birds from eggs injected with de-ionized water (sham).

Effects of *in ovo* injection on organ development and gut morphometry of broiler chickens at day 7 of age

The effects of *in ovo* injection of phytobiotics (garlic, oyster mushroom and their combination) on organ development and gut morphology of broiler chickens at day 7 of age are presented in Table 2. No significant ($P > 0.05$) differences in all organ and gut morphological parameters measured were detected.

Effects of *in ovo* injection on organ development and gut morphometry of broiler chickens at day 35 of age

In Table 3, the effects of *in ovo* injection of garlic, oyster mushroom and their combination on organ development and gut morphology of broiler chickens at day 35 of age showed significant ($P < 0.05$) differences in heart and spleen. The highest proportion of heart (0.65 %) was recorded in birds from eggs injected with oyster mushroom and the least (0.43 %) in those obtained in the control. Meanwhile, the highest proportion of spleen (0.18 %) was observed in birds from eggs injected with the combination of garlic and oyster mushroom, and the lowest (0.07 %) in chicks from eggs subjected to *in ovo* injection of oyster mushroom extract.

Effects of *in ovo* injection on haematological and serum biochemical indices of broiler chickens at day 7 of age

The effects of *in ovo* injection of garlic, oyster mushroom and their combination on haematological and serum biochemical indices of broiler chickens at day 7 of age are shown in Table 4. Significant ($P < 0.05$) difference was observes only in lymphocyte count. Birds from eggs on *in ovo* injection of garlic, the combination of garlic and oyster mushroom, and sham had the highest proportion of lymphocytes (85.50 %) while birds from the control group showed the lowest proportion (80.50 %) comparable to 80 % birds from the *in ovo* injection of oyster mushroom. No significant ($P > 0.05$) differences were detected in serum biochemical indices of the birds.

Effects of *in ovo* injection on haematological and serum biochemical indices of broiler chickens at day 35 of age

In Table 5, there were no significant ($P > 0.05$) differences in all determined parameters concerning the effects of *in ovo* injection of garlic, oyster mushroom and their combination on haematological and serum biochemical indices of broiler chickens at day 35 of age.

Effects of *in ovo* injection on cell-mediated and humoral immune responses of broiler chickens

Table 6 shows no significant ($P > 0.05$) variations in the treatment groups concerning the effects of *in ovo* injection of garlic, oyster mushroom and their combination on cell-mediated and humoral immune responses of the broiler chickens.

Effects of *in ovo* injection on growth performance of broiler chickens

The effects of *in ovo* injection of garlic, oyster mushroom and their combination on growth performance of broiler chickens are shown in Table 7. The final weight, daily weight gain and feed conversation ratio were sig-

Table 1. Effects of *in ovo* injection on hatching traits of broiler chickens

Parameters	In ovo treatments						
	Control	Garlic	Oyster mushroom	Garlic + Oyster mushroom	Sham	SEM	P-Value
Chick weight [g]	45.96^{ab}	50.91^{a}	45.81^{ab}	49.27^{ab}	44.38^{b}	1.58	0.02
Chick: Egg	1.46	1.29	1.47	1.30	1.43	0.05	0.06
Hatchability [%]	87.50	85.00	75.00	85.00	75.00	5.92	0.44
Dead in germ [%]	5.00	2.50	10.00	0.00	12.50	2.29	0.141
Dead in shell [%]	7.50	12.50	15.00	15.00	12.50	4.64	0.80

Sham – positive control; SEM – Standard error of means

Table 2. Effects of *in ovo* injection on gut morphometry of broiler chickens at day 7 of age

	In ovo treatment						
Parameters	**Control**	**Garlic**	**Oyster mushroom**	**Garlic+Oyster mushroom**	**Sham**	**SEM**	**P-value**
Live weight [g.bird-1]	128.50	124.00	115.00	120.00	113.50	5.21	0.34
Organs							
Heart [%]	0.85	1.26	0.84	0.75	0.95	0.20	0.51
Liver [%]	4.17	3.59	3.43	5.02	3.95	0.64	0.51
Proventriculus [%]	0.88	0.83	0.98	0.84	0.96	0.08	0.65
Gizzard [%]	4.34	3.39	4.49	3.67	3.82	0.27	0.14
Gut							
Duodenum [%]	2.03	1.60	2.33	1.98	1.85	0.18	0.21
Duodenum [cm.100g^{-1} LW]	11.67	12.14	13.86	12.08	12.35	0.66	0.30
Pancreas [%]	0.17	0.17	0.17	0.15	0.13	0.02	0.84
Pancreas [cm.100g^{-1} LW]	4.66	4.45	4.75	4.75	4.44	0.49	0.66
Jejunum [%]	3.16	2.12	3.18	2.71	2.82	0.28	0.19
Jejunum [cm.100g^{-1} LW]	26.85	16.65	25.15	24.38	24.00	2.32	0.13
Ileum [%]	2.58	2.74	3.16	2.51	2.25	0.33	0.48
Ileum [cm.100g^{-1} LW]	26.46	23.60	24.55	24.16	22.69	2.21	0.80
Caecum [%]	1.05	1.01	1.44	1.57	1.41	0.33	0.70
Caecum [cm.100g^{-1} LW]	8.95	10.50	10.00	10.60	11.30	0.82	0.44
Colon [%]	0.73	0.39	0.63	0.44	0.49	0.10	0.27
Colon [cm.100g^{-1} LW]	2.33	2.64	2.61	2.08	2.41	0.17	0.29

Sham – positive control; SEM – Standard error of means; LW – Live weight

nificantly ($P < 0.05$) influenced. The highest final weight (2000 g.bird^{-1}) was recorded in birds from eggs on the *in ovo* injection of de-ionized water followed by birds from eggs injected with garlic (1946.70 g.bird^{-1}), the lowest values were observed in birds on the combination of garlic and oyster mushroom (1690.20 g.bird^{-1}). Meanwhile, daily weight gain (55.67 g.bird^{-1}) was the highest ($P < 0.05$) in birds from eggs injected with deionized water (sham) but statistically similar to that of birds from eggs on *in ovo* injection of garlic (54.33 g.bird^{-1}) while the lowest proportion was recorded in birds from eggs on *in ovo* injection of the combination of garlic and oyster mushroom (47.10 g.bird^{-1}). Birds from the sham group had the best FCR (1.68), followed by birds from eggs *in ovo* injected oyster mushroom (1.82) and those from eggs on garlic (1.86) while birds from *in ovo* injection of the combination of garlic and oyster mushroom showed the poorest FCR (2.21).

Table 3. Effects of *in ovo* injection on organ development and gut morphometry of broiler chickens at day 35 of age

Parameters	In ovo treatments						
	Control	Garlic	Oyster mushroom	Garlic+Oyster mushroom	Sham	SEM	P-value
Live weight [g.bird^{-1}]	1465.0	1340.0	1415.0	1195.0	1510.0	87.50	0.23
Organs [%]							
Heart	0.43^{b}	0.49^{ab}	0.65^{a}	0.51^{ab}	0.54^{ab}	0.03	0.03
Liver	2.14	2.31	1.85	2.50	2.86	0.40	0.53
Bursa	0.17	0.33	0.25	0.23	0.20	0.04	0.22
Proventriculus	0.41	0.21	0.39	0.54	0.37	0.07	0.50
Spleen	0.08^{b}	0.13^{ab}	0.07^{b}	0.18^{a}	0.12^{ab}	0.01	0.01
Gizzard	2.05	2.29	2.01	2.18	1.88	0.23	0.68
Gut [%]							
Duodenum [%]	1.37	1.45	1.01	1.31	1.40	0.10	0.15
Duodenum [cm.100g^{-1} LW]	2.11	2.29	1.70	1.94	1.96	0.21	0.45
Jejunum [%]	2.85	2.48	2.54	2.32	2.01	0.44	0.75
Jejunum [cm.100g^{-1} LW]	5.19	5.78	6.01	6.33	4.84	0.68	0.57
Ileum [%]	2.58	2.04	2.17	2.88	1.80	0.38	0.39
Ileum [cm.100g^{-1} LW]	5.07	5.28	5.76	6.21	4.98	0.44	0.36
Caecum [%]	0.78	0.95	0.67	0.65	0.90	0.11	0.37
Caecum [cm.100g^{-1} LW]	2.29	2.80	2.72	2.86	2.37	0.17	0.20
Colon [%]	0.26	0.23	0.20	0.16	0.19	0.04	0.63
Colon [cm.100g^{-1} LW]	0.35	0.44	0.58	0.54	0.52	0.08	0.43
Thymus [%]	0.41	0.29	0.39	0.51	0.42	0.14	0.88

^{a,b} – Means in the same row having different superscript differ significantly ($P < 0.05$); Sham – positive control; SEM – Standard error of means; LW – Live weight

DISCUSSION

The *in ovo* techniques among other advantages seek to jump the start growth and engender early gut development for improved post-hatch growth performance of broiler chickens [29, 37]. *In ovo* injection of aqueous extracts of garlic increased chick weight which could be a result of the growth-promoting, anti-bacterial and anti-parasitic properties of garlic that enable fast growth and prevent the potential negative effects of pathogenic organisms. This result is in line with the findings [7, 10] that before emergence the embryo naturally consumes supplemental nutrients and the chick weight increases with injection of nutrient-based phytogenics. This implies that *in ovo* injections of garlic, and the combination of garlic and oyster mushroom influenced weight gain in this study owing to the fact that extracts of garlic can increase growth and improve feed conversion ratio [8] while mushrooms, being rich in protein, fibre, carbohydrates, vitamins, and minerals can increase chick weight by supporting the growth rate [5].

The digestive system of chicks undergoes a number of morphological changes such as increase in length and density of intestinal villi [36]. These changes are enhanced by many factors including feeding. Birds from eggs subjected to *in ovo* injection of oyster mushroom had the biggest proportion of heart in contrast to the lowest proportion in control birds The highest proportion of spleen was recorded in birds from eggs on *in ovo* injection of extracts of garlic and oyster mushroom and the lowest in birds from oyster mushroom-injected eggs. The increased proportion of heart and spleen observed in this study might be due to the inherent properties of oyster mushroom [5] and, possibly, protective properties of garlic against invasion of microbes [14] which could have enhanced the development of the

Table 4. Effects of *in ovo* injection on haematological and serum biochemical indices of broiler chickens at day 7 of age

Parameters	In ovo treatments						
	Control	Garlic	Oyster mushroom	Garlic+Oyster mushroom	Sham	SEM	P-value
Haematological indices							
Packed cell volume [%]	27.00	27.00	22.50	27.50	28.50	3.25	0.73
Haemoglobin [g.dl^{-1}]	8.96	8.63	7.46	9.13	9.46	1.08	0.74
Red blood cells (×10^{6}.mm^{-3})	2.55	2.55	1.85	2.40	2.45	0.30	0.50
White blood cells [×10^{6}.mm^{-3}]	16.50	16.50	17.50	17.00	16.70	1.62	1.00
Heterophils [%]	13.50	10.50	13.50	10.50	10.50	0.81	0.08
Lymphocytes [%]	80.50b	85.50a	82.00ab	85.50a	85.00a	0.74	0.01
Eosinophils [%]	1.50	1.00	1.00	1.50	1.00	0.31	0.60
Monocytes [%]	4.50	3.00	4.00	3.50	3.50	0.40	0.21
Serum biochemical indices							
Total protein [mg.dl^{-1}]	3.50	2.95	3.25	3.60	3.20	0.19	0.25
Albumin [mg.dl^{-1}]	1.35	1.40	1.20	1.30	1.40	0.22	0.96
Globulin [mg.dl^{-1}]	2.15	1.55	2.05	2.30	1.80	0.33	0.60
Triglyceride [mg.dl^{-1}]	77.50	128.90	145.30	117.30	70.30	37.70	0.60
Cholesterol [mg.dl^{-1}]	157.90	198.20	212.40	182.80	183.40	37.10	0.90
Low-density lipoprotein [mg.dl^{-1}]	129.40	152.70	167.60	137.80	149.60	28.40	0.90
High-density lipoprotein [mg.dl^{-1}]	12.95	19.63	15.71	21.55	19.64	2.44	0.23
Very low-density lipoprotein [mg.dl^{-1}]	15.51	25.77	29.05	23.45	14.06	7.54	0.60
Creatinine [mg.dl^{-1}]	0.61	1.23	1.23	1.64	0.82	0.49	0.65

^{a,b} – Means in the same row having different superscript differ significantly ($P < 0.05$); Sham – positive control; SEM – Standard error of means

immune organ (spleen) in the chickens. In contrast, U n i et al. [33] reported no significant differences in heart and spleen of broiler chicken on *in ovo* injection. This could be ascribed to the mode of administration and the differences in strains of broiler chickens.

The results of haematology and serum biochemical parameters are usually used to assess the health status of an animal as haematological and serum biochemical parameters have served as indicators of their physiological status and their changes reflected response of animals to various physiological situations [13]. The lymphocyte counts differed significantly with the highest values after injection of garlic and the combination of garlic and oyster mushroom, attributable to the immuno-modulating properties of garlic triggering production of higher levels of white blood cells [22]. The normal levels of total protein and albumin in poultry blood are in the range of 3.0–4.9 mg.dl^{-1} and 1.17–2.74 g.dl^{-1}, respectively [18]. Higher concentration of albumin usually denotes dehydration while a lower concentration may be due to the liver malfunction due to factors such as malnutrition and infection. In this study, the total protein and albumin in chicks determined at days 7 and 35 of age was within the normal range. Also, the concentration of cholesterol in birds slaughtered at days 7 and 35 of age was normal within the range of 87–192 g.dl^{-1} [18] in the observed groups except for birds from garlic and oyster mushroom-injected eggs at day 7 of age which showed increased levels of cholesterol, largely due to the phytochemical properties of oyster mushrooms [17]. At day 35 of age, lower values were detected in birds from garlic-injected eggs compared to the normal range, attributable to the cholesterol-reducing effects of garlic [1]. Triglycerides which are found in adipose tissues are synthesized from fatty acids, protein and glucose. At days 7 and 35 of age, the triglycerides in all the groups were within the normal range (45.7–172 mg.ml^{-1}) [19].

Table 5. Effects of *in ovo* injection on haematological and serum biochemical indices of broiler chickens at day 35 of age

Parameters	*In ovo* treatments						
	Control	Garlic	Oyster mushroom	Garlic + Oyster mushroom	Sham	SEM	P-value
Haematological indices							
Packed cell volume [%]	32.50	34.50	32.00	31.50	36.00	0.98	0.09
Haemoglobin [g.dl^{-1}]	10.29	9.96	9.96	10.46	11.46	0.35	0.12
Red blood cells [×10^{6}.mm^{-3}]	2.95	2.75	2.85	2.95	3.15	0.10	0.22
White blood cells [×10^{6}.mm^{-3}]	13.00	15.00	12.50	13.50	11.00	0.34	0.94
Heterophils [%]	11.50	12.00	12.00	12.50	12.00	1.14	1.00
Lymphocytes [%]	83.00	84.00	82.00	83.00	82.50	1.63	0.92
Eosinophils [%]	1.50	1.00	2.50	1.00	1.00	0.32	0.07
Monocytes [%]	4.00	3.50	3.50	4.50	4.50	0.63	0.67
Serum biochemical indices							
Total protein [mg.dl^{-1}]	3.10	3.20	3.15	3.45	3.50	0.15	0.34
Albumin [mg.dl^{-1}]	1.20	1.08	1.55	1.64	2.13	0.25	0.14
Globulin [mg.dl^{-1}]	1.90	2.12	1.60	1.81	1.37	0.27	0.43
Cholesterol [mg.dl^{-1}]	90.45	78.20	95.50	89.30	102.75	8.40	0.43
Triglyceride [mg.dl^{-1}]	97.70	92.70	49.10	74.50	80.40	21.2	0.60
Low-density lipoprotein [mg.dl^{-1}]	57.62	47.29	73.20	60.39	72.25	5.85	0.11
High density lipoprotein [mg.dl^{-1}]	13.30	12.37	12.49	14.01	14.42	2.04	0.93
Very low-density lipoprotein [mg.dl^{-1}]	19.53	18.55	9.81	14.90	16.08	4.24	0.57
Creatinine [mg.dl^{-1}]	0.61	0.93	0.55	0.76	0.46	0.09	0.07

Sham – positive control; SEM – Standard error of means

Table 6. Effects of *in ovo* injection on cell-mediated immune response of broiler chickens

Treatments	CMIR [mm]	Humoral immunity
Control	0.38	2.39
Garlic	0.19	3.30
Oyster mushroom	0.15	2.92
Garlic + Oyster mushroom	0.15	3.61
Sham	0.24	2.31
SEM	0.173	0.356
P-value	0.591	0.251

Sham – positive control; SEM – Standard error of means; CMIR – Cell-mediated immune response

Table 7. Effects of *in ovo* injection on performance of broiler chickens at day 35 of age

Parameters	In ovo treatment						
	Control	Garlic	Oyster mush-room	Garlic+Oyster mush-room	Sham	SEM	P-Value
Initial weight [g.bird^{-1}]	46.60	44.86	42.90	42.66	46.60	0.87	0.17
Final weight [g.bird^{-1}]	1720.00^{bc}	1946.70^{a}	1902.90^{ab}	1690.20^{c}	2000.00^{a}	40.26	0.002
Daily weight gain [g.bird^{-1}]	47.78^{bc}	54.33^{a}	53.14^{ab}	47.10^{c}	55.67^{a}	1.14	0.002
Daily feed intake [g.bird^{-1}]	105.21	101.03	96.45	104.72	93.71	6.66	0.68
Feed conversion ratio	2.20^{a}	1.86^{ab}	1.82^{ab}	2.21^{a}	1.68^{b}	0.11	0.03

^{a,b,c} – Means in the same row having different superscript differ significantly ($P < 0.05$); Sham – positive control; SEM – Standard error of means

The cellular and humoral immune responses showed no variations across the groups indicating that *in ovo* injection of garlic, oyster mushroom and their combination could be immunomodulatory but that the birds were not challenged. This result was in line with the report by S o g u n l e et al. [27] who observed no significant differences in broiler chickens injected with PHA-P and SRBC in a study dealing with *in ovo* injection of inorganic salts of zinc, selenium, copper and their combination.

The result of growth performance corroborated the findings [14, 25] that attributed the performance to the phytochemicals properties of garlic and mushrooms which are antimicrobial, antiviral, antiparasitic, and antifungal [16, 21].

CONCLUSIONS

The study allowed us to conclude that *in ovo* injection of aqueous extracts of garlic and oyster mushroom at 0.1 ml per egg can be beneficial for improving chick weight, growth performance and the development of heart and spleen functions in broiler chickens.

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Conflict of interest

The authors hereby declare no conflicting interest.

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