

Effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom on immune response, tibiae morphometry and minerals, and bone marrow histology of broiler chickens

Sogunle, O. M.¹*; Azeez, N. T.¹; Safiyu, K.K.²; Irivboje, O. A.³; Adeyemi, O. A.¹; Wheto, M.⁴; Odutayo, O. J.¹ and Uyeno, Y.⁵.

¹Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

²Department of Animal Production and Livestock Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

³Agricultural Technology Department, The Federal Polytechnic, Ilaro, Ogun State, Nigeria.

⁴Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

⁵Faculty of Agriculture, Shishu University, Minamiminowa, Kamiina, Nagano, Japan.

SUMMARY

This study determined the effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom (AEOM) on immune response, tibiae morphometry and minerals, and bone marrow histology of broiler chickens. Four hundred fertile eggs from Arbor acre broiler strain were procured, fumigated and weighed. Afterwards, 360 eggs were set into the incubator. On the 14th day of incubation, the eggs were candled and a total of 273 eggs (75.83 % fertility) showing viable embryo were distributed into three groups for *in ovo* administration of AEOM which was carried out on 18th day of embryonic age. Each group was allotted 91 eggs. A total of one hundred and ninety one (191) day-old chicks hatched after incubation; 83 chicks hatched from control (91.20% hatchability), 58 chicks from group 2 (63.74%) and 50 hatched from group 3 (54.95% hatchability). The chicks from groups that had received AEOM via *in ovo* route were orally given supplemental administration of AEOM immediately after hatch. Therefore, resulting in three treatments; T1 (control), T2 (0.1 ml *in ovo* + 0.1 ml posthatch AEOM) and T3 (0.2ml *in ovo* + 0.2 ml posthatch AEOM). The birds were allotted into 5 replicates of 10 birds per replicate and reared for 8 weeks. Data were subjected to One-Way Analysis of Variance in a Completely Randomized Design. Birds in T2 recorded significantly ($P<0.05$) highest PCV and Hb (35.00% and 11.70 g/dl, respectively). Basophil count (1.00) was higher ($P<0.05$) in birds on T3. Calcium content of tibiae was significantly ($P<0.05$) highest (27.56%) in birds on T1. Precursor cells were present in the bone marrow histology of broiler chicken on T2 and T3. The study concluded that the administration of AEOM did not impact positively on hatchability but greatly increased formation of blood cells and improved liver function in broiler chicken at 0.1 ml *in ovo* + 0.1 ml posthatch administration of AEOM.

ADDITIONAL KEYWORDS

Aqueous extract of oyster mushroom.

Bone histology.

Broiler chickens.

In ovo feeding.

Efecto de la administración *in ovo* y post-eclosión de extracto acuoso de hongo ostra sobre la respuesta inmune, la morfometría y minerales de las tibias, y la histología de la médula ósea de pollos de engorde

RESUMEN

Este estudio determinó el efecto de la administración *in ovo* y posteclosión de extracto acuoso de hongo ostra (AEOM) sobre la respuesta inmune, la morfometría de tibias y minerales, y la histología de la médula ósea de pollos de engorde. Cuatrocientos huevos fértiles de la cepa de pollo de engorde Arbor acre fueron adquiridos, fumigados y pesados. Posteriormente, se colocaron 360 huevos en la incubadora. En el día 14 de incubación, los huevos fueron velados y un total de 273 huevos (75,83 % de fertilidad) que mostraban embriones viables se distribuyeron en tres grupos para la administración *in ovo* de AEOM que se llevó a cabo el día 18 de edad embrionaria. A cada grupo se le asignaron 91 huevos. Un total de ciento noventa y un (191) polluelos de un día de edad eclosionaron después de la incubación; 83 pollitos nacieron del control (91,20% de incubabilidad), 58 pollitos del grupo 2 (63,74%) y 50 nacieron del grupo 3 (incubabilidad del 54,95%). Los polluelos de grupos que habían recibido AEOM por vía *in ovo* se les administró oralmente AEOM inmediatamente después de la eclosión. Por lo tanto, resultando en tres tratamientos; T1 (control), T2 (0,1 ml *in ovo* + 0,1 ml posthatch AEOM) y T3 (0,2ml *in ovo* + 0,2 ml posthatch AEOM). Las aves fueron asignadas en 5 réplicas de 10 aves por réplica y criadas durante 8 semanas. Los datos fueron sometidos a un análisis unidireccional de la varianza en un diseño completamente aleatorizado. Las aves en T2 registraron significativamente ($P<0,05$) PCV y Hb más altos (35,00% y 11,70 g/dl, respectivamente). El recuento de basófilos (1,00) fue mayor ($P<0,05$) en aves con T3. El contenido de calcio de las tibias fue significativamente ($P<0,05$) más alto (27,56%) en las aves con T1. Las células precursoras estaban presentes en la histología de la médula ósea del pollo de engorde en T2 y T3. El estudio concluyó que la administración de AEOM no tuvo un impacto positivo en la incubabilidad, pero aumentó en gran medida la formación de células sanguíneas y mejoró la función hepática en pollos de engorde a 0,1 ml *in ovo* + 0,1 ml después de la administración de AEOM.

PALABRAS CLAVE

Extracto acuoso de hongo ostra.

Histología ósea.

Alimentación *in ovo*.

Pollos de engorde.

INFORMATION

Cronología del artículo.

Recibido/Received: 28.11.2020

Aceptado/Accepted: 11.06.2021

On-line: 15.07.2021

Correspondencia a los autores/Contact e-mail: :

sogunleom@funaab.edu.ng

INTRODUCTION

At hatch, broiler chicks mostly suffer delayed intake of water and nutrients from feeds and this results in reduced overall post-hatch performance (Noy Geyra and Sklan 2001, pp.914-7; Gonzales et al. 2003, pp. 472-6; Latour et al. 2003, pp. 1364-7) and increased mortality (Willemsen et al. 2010, pp. 192-7). The delay which is sometimes more than 36 h is often occasioned by hatchery processing (vent-sexing and vaccination), and transportation (Obun & Osaguona 2013, p. 6). Accordingly, Noy & Sklan (1999, p. 21) reported that post-hatch deprivation of feed and water for 48 -72 h reduced body weight of broilers by 7.8% over those fed immediately after hatch. Therefore, early feeding strategies including *in ovo* feeding to specially designed post-hatch diets have been developed to possibly reverse the negative effects of delayed feeding (Uni & Ferket 2004, pp. 104-8; Leeson 2008, pp. 317-9).

It is noteworthy that the earliest recommendations for providing feed at hatch in hatching trays (Sklan et al. 2000, pp. 144-6) was fraught with problems due to tight regulation of environmental factors in the hatchery. Though, feeding immediately post-hatch was shown to be highly beneficial but *in ovo* feeding of nutrients was considered a more effective option (Bhuiyan et al. 2011, pp.1003-5) to jump-start growth by supporting growing embryos with different nutrients and medications (Sharma & Burmester 1982, pp. 135-9; Coşkun et al. 2014, p. 48). The *in ovo* technique is an automated system that punches a small hole through the egg shell into the air cell of the egg to deliver nutrients and/or drugs for the developing embryo through the hole on day 18 of the bird's 21-day incubation period.

To further achieve great success in the broiler industry, Jha et al. (2019, p. 82) stated that nutritionally balanced-feeding programs along with the use of antibiotic growth promoters (AGP) have played significant roles. However, the poultry industry is currently redefining its nutrition program to grow safe and quality meat in the light of public health concern due to uncontrolled use of AGP and synthetic drugs. Prominent alternatives to AGP in poultry production include the use of phytochemical substances such as extracts (Dhama et al. 2014, pp. 130-45; Cimrin et al. 2020, e20190270) from plants; oyster mushroom, garlic, ginger to mention but a few.

Oyster mushroom (*Pleurotus ostreatus*) possesses antimicrobial, antiviral and anticancer properties (Toghyani et al. 2012, pp. 185-7; Sogunle et al. 2019, pp. 26-8, Sert & Ayasan 2020, pp. 66-8). Oligosaccharides components in oyster mushroom are found to contribute to the favourable effects of the phytobiotics on growth (Xue & Meng 1996, pp. 15-7). Although the exact mechanism is not clear but it might be due to the presence of various hepatoprotective substances present in oyster mushroom which provides health benefits (Hossain et al. 2003, pp. 470-3). Although the use of plant extracts as replacement for antibiotics is not new in the poultry industry, but combining both *in ovo* and post-hatch routes for administration of aqueous extract of oyster mushroom to modulate the immunity and bone health of poultry are yet to be studied.

Based on the foregoing, this study determined the effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom on immune response and tibia morphometry and minerals, and bone marrow histology of broiler chickens.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The incubation of fertile eggs was carried out in the Hatchery, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta while the resulting chicks were raised in the Poultry Unit of Directorate of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on altitude of 127 m, latitude 7° 13' N and longitude 3° 26' E (Google Map, 2019).

PREPARATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM

Aqueous extract of oyster mushroom was prepared using hot water extraction method as described by Sogunle et al. (2019, p. 25). Five hundred grams (500 g) of fresh oyster mushroom immersed in 1 litre of water was cooked at 57.2°C for twenty (20) minutes. The newly formed extract was cooled and strain-off the mushrooms with the aid of a sieve. The extract was kept in a dark-coloured recipient (to prevent photolysis due to light penetration) and then stored in the refrigerator at -4°C until needed. Thereafter, 10% of the extract was prepared using deionized water.

INCUBATION OF FERTILE EGGS

Four hundred hatching eggs from Arbor acre broiler strain were procured from a reputable breeder farm in Ogun State, Nigeria. The eggs were sorted and a total of 360 hatching eggs resulted. These were fumigated, weighed and set in the incubator. On the 14th day of incubation, the eggs were candled and a total of 273 eggs (75.83 % fertility) showing viable embryo were distributed into three groups (0, 0.1 and 0.2 ml of aqueous extract of oyster mushroom) on the basis of *in ovo* administration of aqueous extract of oyster mushroom. Each group was allotted 91 eggs.

ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM

IN OVO ADMINISTRATION

On 18th day of embryonic age, the eggs in other groups except the control were injected with 0.1 and 0.2 ml aqueous extract of oyster mushroom into the amnion using a 24-gauge hypodermic needle (25 mm long) under laminar flow system, with handling temperature not lower than 35 °C as described by Sogunle et al. (2018, pp. 10-3). Before injection, the site on the broad end of the egg where the small hole was punched was suitably sterilized with 30% ethanol. After the *in ovo* administration of aqueous extract of oyster mushroom, the injection site was sealed with sterile paraffin and the eggs were transferred to hatching compartment. The *in ovo* injection of each treatment was completed within 30 minutes of taking out the fertile eggs from the incubator.

POST-HATCH ADMINISTRATION AND CHICKS MANAGEMENT

A total of one hundred and ninety one (191) day-old chicks hatched after incubation; 83 chicks hatched from control (91.20% hatchability), 58 chicks from group 2 (63.74%) and 50 hatched from group 3 (54.95% hatchability). The chicks from groups that had been administered oyster mushroom via *in ovo* route were orally given supplemental post-hatch administration (0.1 and 0.2 ml, respectively) of aqueous extract of oyster mushroom immediately after hatch. Afterwards, chicks were allotted into 5 replicates of 10 birds per replicate. Commercial broiler diet and drinking water were provided *ad libitum* throughout the entire rearing period of 8 weeks.

DATA COLLECTION

CELL-MEDIATED IMMUNE RESPONSE

The cell-mediated immune response to phytohemagglutinin type P (PHA-P) was carried out per replicate using the method described by Sogunle et al. (2018, pp. 10-3). At 21 days post-hatch, 0.1 ml (concentration 1 mg.ml⁻¹) of PHA-P was injected into the 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. Cell-mediated immune response was calculated as related by Sogunle et al. (2018, pp. 10-3).

BLOOD PARAMETERS

On the 56th day post-hatch, 3 ml of blood was collected from the brachial vein of 2 birds per replicate into heparinised tubes. All samples were collected in the morning before feeding (between 07:00 am to 09:00 am). Blood collection tubes were kept on ice in cool containers and transported to the laboratory within 2 hours of blood withdrawal. Haematological parameters were determined using the procedures of Sood (2016, p. 100). Packed Cell Volume (PCV) was determined using microhaematocrit capillaries. Haemoglobin concentration (Hb) was determined using cyanmethaemoglobin method which involves mixing 5 ml of Drabkin's solution (1000 ml of deionised water was mixed with 400 mg of Potassium ferricyanide, 280 mg of Potassium dihydrogen phosphate, 100 mg of Potassium cyanide and 1 ml of non-ionic detergent) with 20 µl of blood sample. The mixture was read in a photocolourimeter at 540 nm (green filter). Blood counts were determined using the improved Neubauer's chamber (area of 9 sq/mm and depth of 0.1 mm). Moreover, serum biochemical parameters (Total protein, albumin, globulin, cholesterol, triglycerides, Alkaline Phosphatase (ALP), Alanine transaminase (ALT) and Aspartate transaminase (AST)) were analyzed using commercially available test kits by Randox laboratories, United Kingdom (Model BT294QY).

Tibia morphometry and Determination of tibia minerals (Calcium and Phosphorus)

On the 56th day post-hatch, two birds per replicate were selected and sacrificed through cervical dislocation as described by Sogunle et al. (2018, pp. 10-3). Right tibiae from carcasses were removed for morphometric and mineral composition analyses. Tibiae weights were measured using scientific sensitive scale. The length, proximal and distal width as well as mid shaft width of tibiae were measured with Vernier callipers. Afterwards, each tibia was defatted for 16 hours in petroleum ether (boiling point of 60-80 °C), dried and weighed before ashing in a muffle furnace. The samples were digested with diluted hydrochloric acid (1:2) and mineral extract were prepared according to AOAC (1995). The extract from each replicate were selected and the concentration of Ca and P were determined by Inductively Coupled Plasma Optical Emission Spectrometry (Sogunle et al. 2018, pp. 10-3).

BONE MARROW HISTOLOGY

Bone marrow from left tibiae of the sacrificed birds from each replicate were fixed and stored in 10% neutral buffered formalin. Each of the samples was embedded in paraffin, and a 5-µm section of each sample was placed on a glass slide and stained with haematoxylin and eosin for examination under a light microscope as described by Glick & Rosse (1981, pp. 472-6).

STATISTICAL ANALYSIS

Data generated were subjected to one-way Analysis of Variance in a Completely Randomized Design. Significantly ($p < 0.05$) different means were separated using Tukey test as contained in Minitab® version 17.1.0 (Minitab, 2013).

The model of the study is as follows;

$$Y_{ij} = \mu + A_i + \epsilon_{ij}$$

Where:

Y_{ij} = Individual Observation

μ = Overall mean

A_i = Effect of Factor A (*in ovo* + post hatch administration of Oyster Mushroom)

ϵ_{ijk} = Experimental error

RESULTS AND DISCUSSION

EFFECT OF *IN OVO* AND POST-HATCH ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM ON CELL-MEDIATED IMMUNITY OF BROILER CHICKENS

Table I shows the effect of *in ovo* and post-hatch administration of AEOM on cell-mediated immunity of broiler chickens. There was no significant effect of the treatments on cell-mediated immunity of broiler chickens. Though, it was established (Yang & Feng, 1998; Willis, Isikhuemhen and Ibrahim 2007, pp. 1857-8) that substances in mushrooms such as polysaccharides, glycosides, alkaloids, volatile oils, and organic acids are responsible for regulating the immune responses. The result observed in this study contradicted the report of Xue & Meng (1996, p.16-7) that poly and oligosaccharides present in mushroom affected both innate and adaptive immunity, including cellular and humoral

Table I. Effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom (AEOM) on cell-mediated immunity of broiler chickens (Efecto de la administración in ovo y post-eclosión de extracto acuoso de hongo ostra (AEOM) sobre la inmunidad mediada por células de pollos de engorde).

Treatment	Cell-mediated immune response
Control	0.11
0.1 ml in ovo + 0.1 ml post-hatch AEOM	0.17
0.2 ml in ovo + 0.2 ml post-hatch AEOM	0.10
SEM	0.04
P value	0.584

SEM = Standard error of means

responses. The quantity and frequency of extract administered could be responsible for variation in results.

EFFECT OF *IN OVO* AND POST-HATCH ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM (AEOM) ON HAEMATOLOGICAL PARAMETERS OF BROILER CHICKENS

The effect of *in ovo* and post-hatch administration of AEOM on haematological parameters of broiler chickens is presented in **Table II**. The study revealed significant ($P < 0.05$) differences in PCV, Hb and basophils. Birds administered 0.1 ml *in ovo* + 0.1 ml post-hatch of AEOM recorded significantly ($P < 0.05$) highest PCV and Hb (35.00% and 11.70 g/dl, respectively). Though the values obtained fell within the normal reference range for domestic chickens (Jain 1986, p. 285). The finding corroborated the earlier reports of Abdalla et al. (2009, pp. 252-8), where significant increase in haemoglobin and packed cell volume were observed in mushroom-treated birds.

The authors also reported increased haemoglobin concentration in broiler chickens supplemented with β -D-glucan in the diet. In another study on cockerels, Sogunle et al. (2019, pp. 26-8) reported PCV and Hb were among the haematological indices that differed significantly across treatments on varying inclusion levels of oyster mushroom. Basophil count measured in this study was significantly ($P < 0.05$) higher (1.00) in birds administered

0.2 ml *in ovo* + 0.2 ml post-hatch of AEOM which could indicate that administering oyster mushrooms to broiler chickens aids the formation of blood cells. Accordingly, Wasser & Weis (1999, pp. 67-92) stated that the presence of biologically active substances from higher basidiomycetes of mushrooms stimulates haematogenesis.

EFFECT OF *IN OVO* AND POST-HATCH ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM (AEOM) ON SERUM BIOCHEMICAL PARAMETERS OF BROILER CHICKEN

In **Table III**, the serum biochemical parameters revealed that broiler chickens administered AEOM had reduced AST levels when compared with the control group. AST was significantly ($P < 0.05$) higher in birds on the control treatment and lower birds on 0.1 ml and 0.2 ml *in ovo* and post-hatch administration of AEOM. This indicates that the use of oyster mushroom improved the condition of the liver of the birds. This is in line with earlier reports by Yogeswari, Murugesan and Jagadeeswaran (2012, p. 105) that mushroom have hepatoprotective effect to combat aflatoxin-induced hepatotoxicity in broiler chickens. In another study on the hepatoprotective effect of oyster mushrooms against Paracetamol-Induced liver damage in Wistar Albino Rats, serum AST and ALT levels were significantly lower in groups given pre-treated mushrooms when compared to those of only paracetamol treated groups (Sumy, Jahan and Sultana 2010, pp. 47-9).

On the other hand, the use of aqueous extract of oyster mushroom did not affect other serum biochemical parameters measured thereby negating the findings of previous studies (Khan 2010, pp. 2-8; Daneshmand et al. 2011, pp. 92-5; Deepalakshmi & Mirunalini 2014, pp. 719-23; Sogunle et al. 2019, pp. 26-8) where the effect of mushroom extract significantly influenced serum biochemical indices.

EFFECT OF *IN OVO* AND POST-HATCH ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM (AEOM) ON TIBIAE MORPHOMETRY AND MINERALS (CA AND P) OF BROILER CHICKENS

The effect of combining *in ovo* and post-hatch administration of AEOM on tibiae morphometry and mine-

Table II. Effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom (AEOM) on haematological parameters of broiler chickens (Efecto de la administración in ovo y post-eclosión de extracto acuoso de hongo ostra (AEOM) sobre los parámetros hematológicos de pollos de engorde).

Parameters	Control	0.1 ml in ovo + 0.1 ml post-hatch AEOM	0.2 ml in ovo + 0.2 ml post-hatch AEOM	SEM	P value
Packed cell volume (%)	33.00 ^{ab}	35.00 ^a	30.50 ^b	0.65	0.036
Haemoglobin concentration (g/dl)	11.00 ^{ab}	11.70 ^a	10.15 ^b	0.23	0.038
Red blood cell count ($\times 10^3/\mu\text{l}$)	2.700	2.85	2.55	0.21	0.650
White blood cell counts ($\times 10^3/\mu\text{l}$)	11.20	11.55	9.70	0.72	0.296
Heterophil (%)	37.00	33.00	28.50	3.93	0.420
Lymphocytes (%)	62.50	64.50	68.00	3.49	0.588
Eosinophil (%)	0.50	0.50	1.00	0.71	0.854
Basophil (%)	0.00 ^b	0.00 ^b	1.00 ^a	0.00	<0.000
Monocyte (%)	0.00	2.00	1.50	0.65	0.221

^{ab} Means on the same row having different superscript are significantly ($p < 0.05$) different; SEM = Standard error of means.

Table III. Effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom (AEOM) on serum biochemical parameters of broiler chickens (Efecto de la administración *in ovo* y post-eclosión de extracto acuoso de hongo ostra (AEOM) sobre los parámetros bioquímicos séricos de pollos de engorde).

Parameters	Control	0.1 ml <i>in ovo</i> + 0.1 ml post-hatch AEOM	0.2 ml <i>in ovo</i> + 0.2 ml post-hatch AEOM	SEM	P value
Total protein (g/dl)	5.95	5.55	6.70	1.14	0.786
Albumin (g/dl)	3.35	3.05	4.05	0.54	0.491
Globulin (mg/dl)	2.60	2.50	2.65	0.63	0.985
Cholesterol (mg/dl)	117.0	102.0	106.5	12.5	0.713
Triglycerides (mg/dl)	104.50	82.00	82.50	6.01	0.123
Alkaline Phosphatase (U/L)	22.00	22.50	25.00	3.23	0.795
Aspartate transaminase (U/L)	72.00 ^a	54.00 ^b	47.00 ^b	2.65	0.014
Alanine transaminase (U/L)	36.00	31.00	34.00	2.38	0.434

^{ab} Means on the same row having different superscript are significantly ($p < 0.05$) different; SEM = Standard error of means.

rals (Ca and P) of broilers chickens is shown in **Table IV**. Tibiae morphometric parameters measured were not significantly different ($P > 0.05$) among treatments. This is in agreement with the report by Sogunle et al. (2018, pp. 10-3) where the *in ovo* injection of inorganic salts of Zn, Se, Cu and their combination had negli-

Table IV. Effect of *in ovo* and post-hatch administration of aqueous extract of oyster mushroom (AEOM) on tibiae morphometry and minerals (Ca and P) of broiler chickens (Efecto de la administración *in ovo* y post-eclosión de extracto acuoso de hongo ostra (AEOM) sobre la morfometría de tibias y minerales (Ca y P) de pollos de engorde).

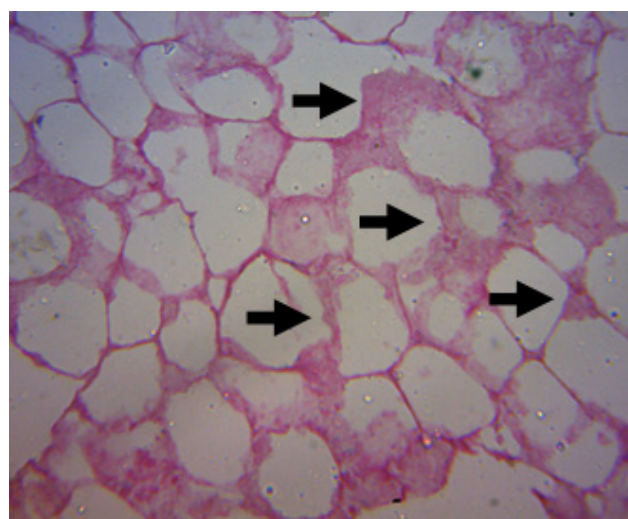
Parameters	Control	0.1 ml <i>in ovo</i> + 0.1 ml post-hatch AEOM	0.2 ml <i>in ovo</i> + 0.2 ml post-hatch AEOM	SEM	P value
Morphometric parameters					
Wet weight (g)	6.59	6.89	5.95	0.40	0.306
Dry weight (g)	3.64	3.58	3.04	0.15	0.050
Length (mm)	67.31	64.63	64.24	1.41	0.317
Proximal width (mm)	16.41	17.09	16.15	0.55	0.502
Distal width (mm)	15.89	16.05	15.50	0.46	0.704
Mid shaft width (mm)	8.73	8.81	8.28	0.31	0.472
Ash weight (g)	1.33	1.34	1.32	0.09	0.992
Minerals					
Calcium	27.56 ^a	23.85 ^{ab}	18.99 ^b	1.60	0.025
Phosphorus	8.84	9.70	11.89	3.02	0.772

^{ab} Means on the same row having different superscript are significantly ($p < 0.05$) different; SEM = Standard error of means.

ble effects on morphometry of tibia bone of broilers. However, calcium content of tibiae was significantly ($P < 0.05$) highest (27.56%) in birds in control treatment and lowest (18.99%) in broiler chickens administered 0.2 ml *in ovo* + 0.2 ml post-hatch of AEOM. This indicates that AEOM reduces the quantity of calcium in the bone. However, limited literatures exist on the impact of plant herbs on bone mineral of poultry.

EFFECT OF *IN OVO* AND POST-HATCH ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM (AEOM) ON BONE MARROW HISTOLOGY OF BROILER CHICKENS

In **Figure 1**, the bone marrow histology of broiler chickens in control is presented. The presence of adipose tissues (arrows) was observed. According to Scheller et al. (2015, p. 7808; 2016, pp. 393-8), the role of adipose tissue in the bone marrow is largely unknown and its morphology and functionality is insufficiently described. However, Hardouin, Rharass and Lucas (2016, p. 85) revealed bone marrow adipose tissue emerges as a distinct fat depot which directly or indirectly interferes with cells of bone remodeling or hematopoiesis in humans. Cawthorn & Scheller (2017, p. 112), further explained that increase in bone marrow adipose tis-

**Figure 1.** Bone marrow histology of broiler chickens in control group (Magnification 100×) (Histología de la médula ósea de pollos de engorde en el grupo control (Aumento 100×)).

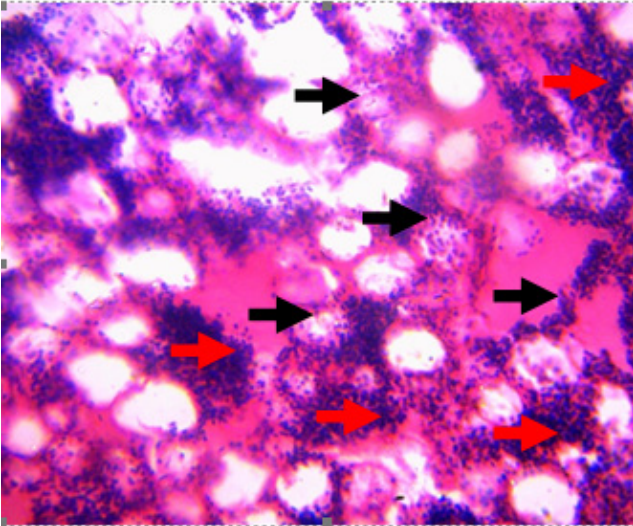


Figure 2. Bone marrow histology broiler chickens administered 0.1 ml *in ovo* + 0.1 ml post-hatch of oyster mushroom extract (Magnification 100×) (Histología de la médula ósea pollos de engorde administrados 0,1 ml en ovo + 0,1 ml después de la eclosión de extracto de hongo ostra (Aumento x100)).

sue often coincides with decreased bone mass, and suggested that bone formation and marrow adiposity are linked. Broiler chickens in the control group of this study were of sound health showing neither signs of severe adiposity nor metabolic disorders. However, a dearth of information still exists on the implication of abundant bone marrow adipose tissues on the health of chicken. Abundant precursor cells (red arrows) were present in the bone marrow histology of broilers administered 0.1 ml *in ovo* + 0.1 ml post-hatch of AEOM (Figure 2). This could greatly explain the highest PCV value recorded in birds on 0.1 ml *in ovo* + 0.1 ml post-hatch AEOM since PCV measures the percentage of red blood cells circulating the blood.

The presence of precursor cells in the bone marrow is an indication of improved immunity since blood

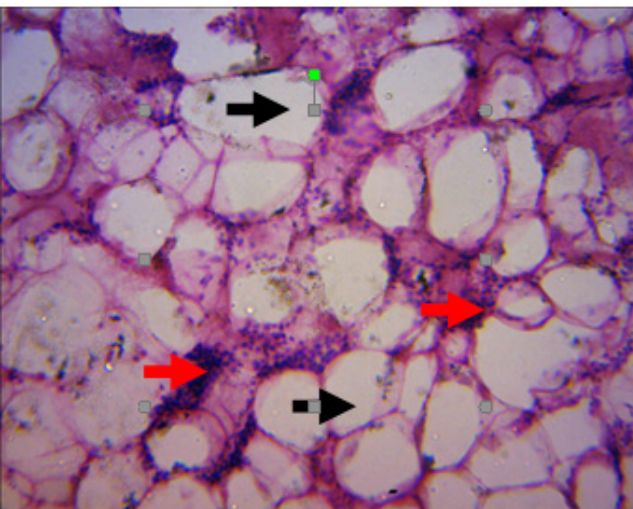


Figure 3. Bone marrow histology of broiler chickens administered 0.2 ml *in ovo* + 0.2 ml post-hatch of oyster mushroom extract (Magnification 100×) (Histología de la médula ósea de pollos de engorde administrados 0,2 ml en ovo + 0,2 ml después de la eclosión de extracto de hongo ostra (Aumento x100)).

cells and its differentials are produced from precursor cells in the bone marrow. There were also few precursor cells (red arrows) within the adipose tissues (black arrows) of bone marrow of broilers administered 0.2 ml *in ovo* + 0.2 ml post-hatch of AEOM in Figure 3.

CONCLUSION AND RECOMMENDATIONS

Therefore, the administration of 0.1 ml *in ovo* + 0.1 ml post-hatch of aqueous extract of oyster mushroom increased formation of blood cells and improved liver function of broiler chickens.

CONFLICT OF INTEREST STATEMENT

There is absolutely no conflict of interest with any individual or organisation regarding the materials discussed in the manuscript.

ACKNOWLEDGEMENT

The Authors wish to appreciate the Centre of Excellence in Agricultural Development and Sustainable Environment of the Federal University of Agriculture, Abeokuta who provided the grant for the execution of this study.

BIBLIOGRAPHY

- Abdalla O, El-Boushy M, Nagwa AS & Halla N 2009. Hematological and biochemical studies on dietary mushroom and oxytetracycline in broiler chicken. *S.C.V.M. Journal*, 514(2): 251-264.
- AOAC 1995. Official methods of analysis, 16th edition. Association of Official Analytical Chemists, Arlington, Virginia, 1995, 22201 USA.
- Bhuiyan MM, Gao F, Chee SH & Iji PA 2011. Minimising weight loss in new broiler hatchlings through early feeding of simple sugars. *Animal Production Science* 51:1002-1007.
- Cawthorn WP & Scheller EL 2017. Editorial: Bone Marrow Adipose Tissue: Formation, Function, and Impact on Health and Disease. *Frontier in Endocrinology* 8:112. doi: 10.3389/fendo.2017.0011
- Cimrin T, Tunca RI, Avsaroglu Md, Ayasan T & Küçükersan S 2020. Effects of an antibiotic and two phytochemical substances (cinnamaldehyde and 1,8-cineole) on yolk fatty acid profile and storage period-associated egg lipid peroxidation level. *Revista Brasileira de Zootecnia*, 49:e20190270.
- Coşkun I, Erenler G, Şahin A, Karadavut U, Altop A & Okur AA 2014. Impacts of In Ovo Feeding of DL-Methionine on Hatchability and Chick Weight. *Turkish Journal of Agriculture - Food Science and Technology*, 2(1): 47-50.
- Daneshmand A, Sadeghi G, Karimi A & Vaziry A 2011. 'Effect of oyster mushroom (*Pleurotus ostreatus*) with and without probiotic on growth performance and some blood parameters of male broilers'. *Animal Feed Science Technology*, 170 (1-2): 91-96.
- Deepalakshmi K & Mirunalini S 2014. '*Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties'. *Journal of Biochemical Technology*, 5(2): 718-726.
- Dhama K, Tiwari R, Khan RU, Chakraborty S, Gopi M, Karthik K, Saminathan M, Desingu PA & Sunkara LT 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances- A Review. *International Journal of Pharmacology* 10: 129-159.
- Glick B & Rosse C 1981. Cellular composition of bone marrow in the chicken: II. The effects of age and the influence of the bursa of Fabricius on size of cellular compartments. *Anat. Rec.*, 200 (4): 471-479.
- Gonzales E, Kondo N, Saldanha SPB, Lody M, Careghi C & Decuyper D 2003. Performance and physiological parameters of broiler

- chickens subjected to fasting on the neonatal period. *Poultry Science*, 82:1250–1256.
- Google map 2019. Federal University of Agriculture. Retrieved from <https://earth.google.com/web/@7.22330744,3.44033719,137.84884575a,1046.69760578d,35y,100.57030218h,44.99999706t,-Or/data=Cm4abBjMciUweDEwM>. Accessed October 2019.
- Hardouin P, Rharass T & Lucas S 2016. Bone Marrow Adipose Tissue: To Be or Not To Be a Typical Adipose Tissue *Frontiers in endocrinology*, 7: 85. doi:10.3389/fendo.2016.00085
- Hossain S, Hashimoto M, Choudhury EK, Alam N, Hussain S & Hasan M 2003. Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. *Clinical and Experimental Pharmacology and Physiology* 30: 470-475.
- Jain NC 1986. Schalm Veterinary Haematology (4th edition). Lea and Febiger, Philadelphia. Pp. 285.
- Jha R, Singh AK, Yadav S, Berrococo JFD & Mishra B 2019. Early Nutrition Programming (*in ovo* and Post-hatch Feeding) as a Strategy to Modulate Gut Health of Poultry. *Frontier in Veterinary Science*, 6: 82.
- Khan MA 2010. 'Nutritional composition and hypocholesterolemic effect of mushroom: *Pleurotus sajor-caju* and *Pleurotus florida*'. LAP Lambert Academic publishing GmbH & co. KG: Saarbrücken, Germany, pp. 1-11
- Khatun K, Mahtab H, Khanam PA, Sayeed MA and Khan KA 2007. Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. *Mymensingh Medical Journal*, 16: 94-99.
- Latour MA, Peebles ED, Boyle CR & Brake JD 2003. The effects of dietary fat on growth performance, carcass composition, and feed efficiency in the broiler chick. *Poultry Science*, 73:1362–1369.
- Lavi I, Friesem D, Geresh S, Hadar Y & Schwarz B 2006, 'An aqueous polysaccharide extract from the edible mushroom (*Pleurotus ostreatus*) induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells'. *Cancer Letters*, 2444: 61-70.
- Leeson S 2008. Predictions for commercial poultry nutrition. *Journal of Applied Poultry Research* 17:315–22.
- MINITAB 17 Statistical Software. 2013. Stable release 17.1.0
- Noy Y, Geyra A & Sklan D 2001. The effects of early feeding on growth and small intestinal development in the post hatch poultry. *Poultry Science*, 80: 912-919.
- Noy Y & Sklan D 1999. Different types of early feeding and performance in chicks and poults. *Journal of Applied Poultry Research*, 8:16-24.
- Obun CO & Osaguona PO 2013. Influence of post-hatch starvation on broiler chick's productivity. *IOSR Journal of Agriculture and Veterinary Science Volume 3, Issue 5 (May - Jun. 2013), PP 05-08*
- Scheller EL, Cawthorn WP, Burr AA, Horowitz MC & MacDougald OA. 2016. Marrow adipose tissue: trimming the fat. *Trends in Endocrinology and Metabolism*, 27:392-403, 10.1016/j.tem.2016.03.016
- Scheller EL, Doucette CR, Learman BS, Cawthorn WP, Khandaker S & Schell B 2015. Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. *Nature Communication*, 6: 7808, 10.1038/ncomms8808
- Sert F & Ayaşan T 2020. Usage Opportunities of Waste Mushroom Composite in Animal Nutrition. *Osmaniye Korkut Ata University Journal of Natural and Applied Sciences*, 3(1): 64-70.
- Sharma J & Burmester B 1982. Resistance of Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. *Avian Diseases* 26:134–149.
- Sklan D, Noy Y, Hoyzman A & Rozenboim I 2000. Decreasing weight loss in the hatchery by feeding chicks and poults in hatching trays. *Journal of Applied Poultry Research* 9:142–148.
- Sogunle OM, Elangovan AV, David CG, Gosh J & Awachat VB 2018. Response of broiler chicken to *in ovo* administration of inorganic salts of zinc, selenium and copper or their combination. *Slovak Journal of Animal Science*, 51 (1): 8–19
- Sogunle OM, Labinjo OS, Olanite JA & Adebowale AA 2019. Growth performance and blood profile of cockerel chickens on administration of oyster mushroom (*Pleurotus ostreatus*) in water and feed. *Archivos de Zootecnia* 68 (261): 24-30
- Sood R 2016. Medical Laboratory Technology: Methods and Interpretations. Sixth edition. Jaypee Brothers Medical Publishers Ltd. New Delhi, India.
- Sumy A, Jahan N, & Sultana N 2010. Study on the Hepatoprotective effect of oyster mushroom (*Pleurotus florida*) Against Paracetamol Induced Liver Damage in Wistar Albino Rats. *Journal of Bangladesh Society of Physiologist*, 5(2): 46-52.
- Toghyani M, Tohid M, Gheisari A, Tabeidian A & Toghyani M 2012. Evaluation of oyster mushroom (*Pleurotus ostreatus*) as a Biological Growth Promoter on Performance, Humoral Immunity, and Blood Characteristics of Broiler Chicks. *Journal of Poultry Science*, 49: 183-190
- Uni Z & Ferket PR 2004. Methods for early nutrition and their potential. *Worlds Poultry Science Journal*. 60:101–111. doi: 10.1079/WPS20038
- Wasser SP & Weis AL 1999. Medicinal properties of substances occurring in higher basidiomycete mushrooms: A modern prospective. *Critical Reviews in Immunology*.19:65–96.
- Willemsen H, Debonne M, Swennen Q, Evereaert N, Careghi C, Han H, Bruggeman V, Tona K & Decuyper E 2010. Delay in feed access and spread of hatch: Importance of early nutrition. *World's Poultry Science Journal*, 66: 189–199.
- Willis WL, Isikhuemhen OS & Ibrahim SA 2007 Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. *Poultry Science*, 86: 1856-1860.
- Xue M & Meng XS 1996. Review on research progress and prosperous of immune activities of bio-active polysaccharides. *Journal of Traditional Veterinary Medicine*, 3: 15-18.
- Yang Y & Feng WS 1998. Brochures of isolation and extraction of chemical components from herbs. China Chinese Herb Medicine Press, Beijing. China.
- Yogeswari R, Murugesan S & Jagadeeswaran A 2012. Hepatoprotective effect of oyster mushroom (*Pleurotus sajor caju*) in Broilers Fed Aflatoxin. *International Journal of Veterinary Science*, 1(3): 104-107.