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

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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 post-hatch AEOM) and T3 (0.2 ml in ovo + 0.2 ml post-hatch 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 post-hatch administration of AEOM.

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 les asignaron 91 huevos. Un total de ciento noventa y un (191) polluelos de un día de edad eclosionaron después de la incubación; 83 polluelos nacieron del control (91,20% de incubabilidad), 58 polluelos del grupo 2 (63,74%) y 50 nacieron del grupo 3 (54,95%). 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 post-hatch administration of AEOM.
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 & Osaguna 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 (Slkan 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 (Bhuyan 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 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 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.

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 to 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 deionized 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 photo-colorimeter 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:

\[ Y_{ij} = \text{Individual Observation} \]

\[ \mu = \text{Overall mean} \]

\[ A_{i} = \text{Effect of Factor A (in ovo + post hatch administration of Oyster Mushroom)} \]

\[ \epsilon_{ij} = \text{Experimental error} \]

**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 1 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell-mediated immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.11</td>
</tr>
<tr>
<td>0.1 ml in ovo + 0.1 ml post-hatch AEOM</td>
<td>0.17</td>
</tr>
<tr>
<td>0.2 ml in ovo + 0.2 ml post-hatch AEOM</td>
<td>0.10</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
</tr>
<tr>
<td>P value</td>
<td>0.584</td>
</tr>
</tbody>
</table>

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 (35.0% 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 hepatoxicity 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 & Murunalini 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 morphology and minerals (Ca and P) of broiler chickens**

The effect of combining in ovo and post-hatch administration of AEOM on tibiae morphology 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).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>0.1 ml in ovo + 0.1 ml post-hatch AEOM</th>
<th>0.2 ml in ovo + 0.2 ml post-hatch AEOM</th>
<th>SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (%)</td>
<td>33.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65</td>
<td>0.036</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/dl)</td>
<td>11.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23</td>
<td>0.038</td>
</tr>
<tr>
<td>Red blood cell count (× 10³/µl)</td>
<td>2.70</td>
<td>2.85</td>
<td>2.55</td>
<td>0.21</td>
<td>0.650</td>
</tr>
<tr>
<td>White blood cell counts (× 10³/µl)</td>
<td>11.20</td>
<td>11.55</td>
<td>9.70</td>
<td>0.72</td>
<td>0.296</td>
</tr>
<tr>
<td>Heterophil (%)</td>
<td>37.00</td>
<td>33.00</td>
<td>28.50</td>
<td>3.93</td>
<td>0.420</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>62.50</td>
<td>64.50</td>
<td>68.00</td>
<td>3.49</td>
<td>0.588</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
<td>0.71</td>
<td>0.854</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>0.00</td>
<td>2.00</td>
<td>1.50</td>
<td>0.65</td>
<td>0.221</td>
</tr>
</tbody>
</table>

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

Archivos de zootecnia vol. 70, número 271, p. 287.
Tibiala 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 negligible 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.

**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).

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

^aMeans on the same row having different superscript are significantly (p<0.05) different; SEM = Standard error of means.

**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 tissue...
EFFECT OF IN OVO AND POST-HATCH ADMINISTRATION OF OYSTER MUSHROOM ON IMMUNE RESPONSE, TIBIAE BONE MARROW HISTOLOGY OF BROILER

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 de pollos de engorde administrados 0.1 ml en ovo + 0.1 ml después de la eclosión de extracto de hongo ostra (Aumento X100)).

Figure 3. Bone marrow histology 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)).

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

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BIBLIOGRAPHY


Cimrin T, Tunca RI, Avsaroglu Md, Ayasan T & Küçükersan S 2020. Effects of an antibiotic and two phytoherapeutic 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


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,1046.89760578d,35y,100.57030218h,44.999999
706t,Or/data=Cm4abBjGm+GueDEwM. 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


MINITAB 17 Statistical Software. 2013. Stable release 17.1.0


