EFFECTS OF *IN OVO* ADMINISTRATION OF AQUEOUS EXTRACT OF OYSTER MUSHROOM AT VARYING LEVELS ON HATCHING CHARACTERISTICS OF BROILER CHICKENS

Oluwabusayo Irivboje & Omobola Olufayo

Department of Agricultural Technology, The Federal Polytechnic, Ilaro.
simbiat.kareem@federalpolyilaro.edu.ng

Abstract

Exogenous nutrients are injected into the amnion of a late-term avian embryo during in ovo feeding in order to promote post-hatch growth, immunological responses, and gastrointestinal tract development. In this study, the effects of aqueous extract oyster mushroom (AEOM) injections at different doses (0, 0.1, and 0.2 mL) on broiler chicken hatching features were examined. For the experiment, a total of 744 viable eggs were used. The eggs were placed in the incubator after being weight-balanced and fumigated. Eggs were candled on the fourteenth day of incubation, and in ovo injection of (AEOM) was given on the eighteenth day. Data collected were subjected to one-way Analysis of Variance in a Completely Randomized Design. The findings indicated that in ovo injection of AEOM considerably ($p<0.05$) altered hatchability %. The 0.2 mL AEOM-injected eggs had the best hatchability (64.58%), while the control (0 mL) eggs had the lowest hatchability (38.41%). The study found that broiler egg hatchability was improved by in ovo injection of AEOM at 0.2 mL/egg.

***Keywords**: In ovo, oyster mushroom, hatching, poultry, broiler*

Background information of the study

Chicken is relatively cheap, rich in nutrients, and acceptable by all culture and religion in Nigeria (Voila *et al.*, 2012). To support its growth, the chicken embryo grows in a definite store of energy and nutrients. Modern fast-growing broiler strains have higher metabolic demands than their forefathers due to their faster growth rate. Poor nutritional status has been demonstrated to increase death rates, growth retardation, and muscle deposition (Halevy *et al.*, 2000). Also it is noteworthy to know that to match the decreased generation interval required for meat birds to reach market size, embryonic development takes up a larger percentage of a bird's life (Sogunle *et al*., 2018).

Since nutrient utilization in chicken starts from the very first day of incubation, where both albumen and yolk nurture developing embryo, it is of utmost importance for the developing embryo to get the proper nutrient supply, since their development or formation occurs in a closed system. The transfer of nutrients from the breeder hen to the embryo is finalized prior to laying of the egg, therefore all required nutrients by the developing embryo for growth and development are contained in the egg (Noy and Uni, 2010). After hatching, the nutrients stored in the yolk in the peritoneal cavity are used up by the chicks (Noy and Uni, 2010). The administration of extrinsic nutrients into the amnion of the late term poultry embryo is termed *in ovo* feeding (Uni et al., 2005). By adding nutrients to the amnion *in ovo* feeding, the perinatal chick's energy status and gut development will be improved since the poultry embryo orally ingest the amniotic fluid (which majorly contains albumen protein and water) before piping the air cell (about 18 days of incubation) (Uni et al., 2005). According to studies, *in ovo* injection is frequently used for a variety of purposes, including fertilising avian eggs inside their shells (Cantrell and Wooten, 2003), administering immunological material into poultry eggs (Jochemsen and Jeurissen, 2002), sex determination of the growing embryo (Kagmi and Hanada, 1997), influencing post-hath body weights of birds by infusing growth promoters into their eggs (Ohta et al., 1999)

Mushrooms have historically been valued for their medical properties, and according to Breene (1990), they are a significant source of bioactive chemicals. Additionally, mushrooms have been said to engage in a variety of behaviours (Guo et al. 2003). Extracts from various mushrooms are of particular interest because they have been shown to have health-promoting effects on farm animals this is largely due to a variety of composites with immune-enhancing, antibacterial, stress-reducing properties and antioxidant (Dalloul and Lillehoj, 2006; Dalloul et al., 2006).

Studies have confirmed the use of *in ovo* feeding of antibiotics, synthetic minerals, vaccines and vitamins in Poultry production (Ferket and Uni, 2006; Mehdi *et al.*, 2011; Nayak et al., 2016) and their resulting effects, but there is paucity of research on the *in ovo* administration of phytogenic properties such as Oyster mushroom in broiler chicken

production. Hence, this research evaluated the effect of these phytogenic substance (oyster mushroom) on the hatching characteristics of broiler chicken eggs.

Materials and Methods

Description of Experimental Location

The research was executed at the Hatchery section, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta.

Source of Fertile Eggs of Broiler Chicken

One thousand (1000) broiler fertile eggs was procured from a creditable Breeder Farm in Ibadan Oyo state.

Hatchery Preparation and Management of Fertile Eggs

The incubator (chick master) that was used consisted of one setter with the capacity to contain 700 eggs and a hatching compartment consisting of 4 hatching trays. The hatching equipment (setting trays, hatching creates, incubator) were cleaned and disinfected to prevent infections from the environment before setting. The incubator was then fumigated with mixture of formaldehyde and potassium permanganate (KMnO4) in a ratio 1:2. The eggs were also fumigated using KMnO4 and formaldehyde in ratio 1:2 for 20 minutes in an enclosure before setting in the incubator. The fertile eggs set (744, 74.4% settable eggs) were managed at appropriate temperature (between 37.5^0C – 37.8^0C), relative humidity (60-65%) and turned manually at every hour for 18 days of incubation. After candling on the 14th of incubation, the eggs with living embryo were then sub-divided into the treatment groups of Control, 0.1 mL and 0.2 mL *in ovo* injection of AEOM to contain 112 fertile eggs each.

Preparation of aqueous extract Oyster mushroom (AEOM) for *in ovo* administration

Fresh Oyster mushroom (Pleurotus ostreatus) were purchased from Ibadan, Oyo State. The oyster mushrooms were thoroughly cleansed to remove any form of dirt on it. After cleaning, hot water extraction procedure was used for mushroom extraction. Five hundred grams (500 g) of Oyster mushroom to 1 litre of water was cooked in a beaker in the laboratory at 57.2^0C for twenty minutes. A sieve was used to separate the mushroom from the freshly generated extracts when they had cooled. Thereafter, 20% aqueous extract Oyster mushroom (20 mL of aqueous extract Oyster mushroom into 80 mL deionized water). A dark-coloured receiver was used to store the freshly generated solution (to avoid photolysis from light penetration). and then refrigerated at -4^0C until needed (Sogunle et al., 2019).

In ovo aqueous extract Oyster mushroom injection

The eggs were injected with 0.1 and 0.2 millilitres of AEOM on the 18th day of embryonic development (after sterilization at 132°C for 4 minutes to prevent contamination) using 24 gauge hypodermic needle (Bhanja et al., 2004).

Data Collection

Data were collected on hatching characteristics during incubation and post-hatching performance during rearing.

Hatching Traits

1. Egg Weight

Eggs obtained from each replicate were weighed respectively before setting in the incubator.

2. Percentage Hatchability

This was evaluated at the end of incubation by dividing no of hatched chicks by no of fertile eggs. As represented below:

$$\text{Hatchability (\%)} = \frac{No\ of\ chicks\ hatched}{No\ of\ fertile\ eggs}\ x100$$

3. Chick Weight (g)

Each chick was weighed at hatch to determine the hatching weight of each chick and divided by the total number of chick hatched per replicate.

$$\text{Average chick weight (g)} = \frac{Total\ weight\ of\ chick\ hatched\ per\ replicate}{Number\ of\ chick\ hatched\ per\ replicate}.$$

4. Egg to Chick Ratio

This was evaluated as the ratio of the chick weight to egg weight.

5. Embryonic Mortality

This was evaluated at the end of the incubation period by collecting all un-hatched eggs in each replicate basis. These were carefully broken to observe the stage of embryonic mortality. Dead in germ percentage was calculated by dividing no of dead in germ per replicate by no of fertile eggs per replicate. As represented below:

Dead in germ (%) = $\frac{No\ of\ dead\ in\ germ\ per\ replicate}{No\ of\ fertile\ egg\ per\ replicate} x\ 100$

Dead in shell percentage was calculated by dividing no of dead in shell per replicate by no of fertile eggs per replicate. As represented below:

Dead in shell (%) = $\frac{No\ of\ dead\ in\ shell\ per\ replicate}{No\ of\ fertile\ egg\ per\ replicate} x\ 100$

Statistical Analysis

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

Results

The table shows the hatchability performance of eggs administered *in ovo* injection of AEOM. According to the findings, *in ovo* injection of AEOM considerably ($p<0.05$) altered the hatchability of broiler eggs. With 0.2 mL injection of AEOM recording similar but higher hatchability percentage (64.58±4.93) and the control group had the least percentage (38.41±5.40). The control group recorded the highest egg weight and chick weight averages, and the average percentage of dead in germ were also found to be substantially ($p<0.05$) different and least percentage of dead in germ (51.361±0.48), (36.23±1.42) and (0.056±0.048), respectively.

Effect of *in ovo* feeding of aqueous extract of Oyster mushroom on hatching traits of broiler chickens

Parameters	*In ovo* feeding			
	Control	0.1 mL	0.2 mL	P-Value
%Fertile Eggs	47.07±1.06	44.28±0.89	45.13±0.89	0.14
No of Hatched chicks	4.30±0.44	3.92±0.39	5.17±0.40	0.09
% Hatchability	38.41±5.40^{b}	49.04±4.74^{ab}	64.58±4.93^{a}	0.00
Av. Egg weight (g)	51.36±0.48^{a}	45.09±0.42^{b}	45.81±0.44^{b}	0.00
Av. Chick weight (g/bird)	36.23±1.42^{a}	33.12±1.24^{ab}	30.76±1.29^{b}	0.03
Egg: chick	1.42±0.12	1.37±0.11	1.62±0.11	0.26
%Dead in Germ	0.06±0.05^{b}	0.23±0.03^{a}	0.25±0.03^{a}	0.01
%Dead in Shell	0.32±0.08	0.39±0.06	0.27±0.06	0.39

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

Discussion

A variety of factors, including brooding and hatching technique, the egg's quality (diameter, permeability, break resistance), the egg's content, and indirectly the welfare, nutrition, and health of the birds, affect how easily poultry eggs hatch (Krawczyk et al., 2012). Plant-based extracts have been proven to be free of residue, natural, and less damaging when compared to the use of conventional inorganic compounds in the production of food animals, and are thus viewed as ideal feed additives (Wang et al., 1998). The findings of this study demonstrate that employing aqueous oyster mushroom extract (AEOM) *in ovo* improved the hatchability of broiler eggs. The maximum hatchability was achieved from eggs on 0.2 mL of AEOM. This may be because to phytochemicals found in oyster mushrooms that have been injected *in ovo*, including 1, 2-Benzenedicarboxylic acid, diisooctyl ester, and 3, 4-Dihydro-2H-pyran. When the eggs were injected at room temperature, these substances also act as antibacterial and antifouling agents. This result is in agreement with the observations made by Ipek et al. (2004) and Al-Daraji et al. (2012), who noted that ascorbic acid and L-arginine injections made eggs more hatchable. The study's hatchability findings show that the injected AEOM solution is safe for growing embryos, as revealed by the 0.2 mL groups. The effect of the extract on the eggs' capacity to hatch was dose-dependent. This is in line with the report of Oke et al. (2021), who saw an improvement in broiler chick hatchability after giving them various doses of black cumin (*Nigella sativa*) extract.

The average egg weight and chick weight in this study were also discovered to be significant. Birds with eggs on the control had greater values than those with eggs on the AEOM injection. The difference in egg set weight could be the cause of the significance seen in chick weight. The weight of chicks upon hatching is greatly influenced by the weight of settable eggs, according to the studies of Ramaphala and Mbajiorgu (2013) and Duman and Sekerolu (2017). In all of the treatments, it was discovered that embryonic mortality was high. Birds that received doses of 0.1 mL and 0.2 mL of AEOM, respectively, had greater rates of dead in germ and dead in shell. The timing of the eggs' removal from the incubator for injection may be to blame for the higher reported embryonic death with 0.1 and 0.2 mL, this is because eggs in the control group was not removed from the setter all through the period. El-Kholy et al.'s (2019) study of significantly varying levels of embryonic mortality on eggs provided water-soluble vitamins to Japanese quail is in agreement with these findings.

Conclusion

The study concluded that *in ovo* administration of Aqueous Extract Oyster Mushroom (AEOM) at 0.2 mL increased percentage hatchability and initial weight of broiler chicks. However, dead in germ was significantly influenced at 0.1 mL and 0.2 mL level of inclusion.

References

Al-Daraji, H.J., Al-Mashadani, A.A. Al-Hayani, W.K. Al-Hassani, A.S. & Mirza. H.A. (2012). Effect of in-ovo injection with L-arginine on productive and physiological traits of Japanese quail. *South African Journal of Animal Science* 42:139-145.

Bhanja, S.K., Mandal, A.B. & Goswami, T.K. (2004). Effect of *in ovo* injection of amino acids on growth, immune response, development of digestive organs and carcass yield of broiler. *Indian Journal of Poultry Science* 2004; 39:212-218.

Breene, W. M. (1990). Nutritional and medicinal value of specialty mushrooms. *Journal of Food Protection* 53: 883–894.

Cantrell, T. & Wooten, A. (2003). Methods of fertilizing an avian egg in the shell. *United State ovo Biosciences, Inc.* (Athens, GA). 657309. http://www.freepatentsonline.com/6573097 htmL.

Dalloul, R. A. & Lillehoj, H. S. (2006). Poultry coccidiosis. Recent advancements in control measures and vaccine development. *Expert Review of Vaccines* 5:143–163.

Dalloul, R. A., Lillehoj, H. S., Lee, J. S., Lee, S. H. & Chung, K. S. (2006). Immunopotentiating effect of a Fomitella fraxinea-derived lectin on chicken immunity and resistance to coccidiosis. *Poultry Science* 85:446–451.

Duman, M. & Şekeroğlu, A. (2017). Effect of Egg Weights on Hatching Results, Broiler Performance and Some Stress Parameters. *Brazilian Journal of Poultry Science*, v.19 / n.2 / 255-262.

El-Kholy, M. S., Ibrahim, Z A., El-Mekkawy, M. M. & Alagawany, M. (2019). Influence of in ovo administration of some water-soluble vitamins on hatchability traits, growth, carcass traits and blood chemistry of Japanese quails. *Ann. Animal Science*, Vol. 19, No. 1 (2019) 97–111

Ferket P & Uni Z. (2006). Early feeding–*in ovo* feeding enhances of early gut development and digestive capacity of poultry.

Guo, F. C., Savelkoul. H. F. J., Kwakkel. R. P., Williams. B. A. & Verstegen, M. W. A. (2003). Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *World's Poultry Science J*ournal 59:427–440.

Halevy, O., Geyra, A., Barak, M., Uni, Z. & Sklan, D. (2000). Early post-hatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. *Journal of Nutrition* 130: 858-864.

Ipek, A., Sahan, U. & Ylmaz, B. (2004). The effect of *in ovo* ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. *Arch. Geflügelk*. 68: 132-135.

Jochemsen, P. & Jeurissen, S. H. M. (2002). The localization and uptake of *in ovo* injected soluble and particulate substances in the chicken. *Poultry Science* 81:1811-1817

Kagmi, H. & Hanada, H. (1997). Current knowledge of sexual differentiation in domestic fowl. *World's Poultry Science Journal* 53:111123.

Krawczyk, J., Puchała, M., & Obrzut, J. (2012). Hatchability in laying hen flocks under the conservation programme (in Polish). *Wiadomości Zootechniczne*, 4: 41–46.

Mehdi, S., Yahya, E., Habib, A., Shahryar & Alireza, L. (2011). The effects of *in ovo* injection of L-threonine in broiler breeder eggs on characters of hatching and growth performance broiler chickens. *European Journal of Experimental Biology*, 2011, 1 (4):164-168, ISSN: 2248 –9215.

Nayak, N., Asha Rajini, R., Ezhilvalavan, S., Sahu, A R. & Kirubaharan, J. J. (2016). Influence of In-ovo Arginine Feeding on Post-hatch Growth Performance and Economics of Broilers. *Journal of Animal Research*: 6(4): 585-591

Noy, Y. & Uni, Z. (2010). Early nutritional strategies. *World's Poultry Science Journal*, 66: 639-646.

Ohta, Y., Tsushima, N., Koide, K., Kidd, M.T. & Ishibashi, T. (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poultry Science* 1999; 78: 1493–1498.

Oke, O. E., Sorungbe, F.O., Abioja, M.O., Oyetunji, O., & Onabajo, A.O. (2016). Effect of different levels of honey on physiological, growth and carcass traits of broiler chickens during dry season. *Acta Agric Slov* 108:45–53

Ramaphala, N. O. & Mbajiorgu, C. A. (2013). Effect of Egg Weight on Hatchability and Chick Hatch-weight of COBB 500 Broiler Chickens. *Asian Journal of Animal and Veterinary Advances*, 8: 885-892.

Sogunle, O. M., Aremu, T. S., Olatubosun, O. O., Njoku, C. P. & Abiona, J. A. (2018). Growth performance and blood profile of rabbit bucks in two housing types on aqueous extract of Oyster mushroom (Pleurotus ostreatus Jacq ex fr.). *Nigerian Journal of Animal Production* 2018, 45(4): Pages 225-233.

Sogunle, O.M., Labinjo, O.S., Olanite, J.A., & Adebowale, A.A. (2019). Growth performance and blood profile of cockerel chickens on administration of Oyster mushroom (*Pleurotus ostreatus*) in water and feed. *Archivos de Zootecnia Journal* 68 (261): 24- 30. 2019.

Uni, Z., Ferket, P., Tako, E. & Kedar, O. (2005). *In ovo* feeding improves energy status of late term chicken embryos. *Poultry Science*, 84: 764-770.

Voila M., & Triches, D. A. (2012). A chicken network: an analysis of Brazilian and world market from 2002 to 2012 (English translation)], *Teoria e Evidência Econômica*, 2015; 21: 126–48. doi: 10.5335/rtee.v21i44.5357.

Wang, R. J., Li, D. F. and Bourne, S. (1998). Can 2000 years of herbal medicine history help us solve problems in the year 2000? Pages 273–291 in *Biotechnology in the Feed Industry*. Proc. Alltech's 14th Annu. Symp. Univ. Pres, Nottingham, UK.