Nig. J. Anim. Prod. 2022, 49(1):131-140. https://doi.org/10.51791/njap.v49i1.3411 *Nigerian Journal of Animal Production*

Influence of *in ovo* administration of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) on post-hatch performance of broiler chickens

[*]Sogunle, O. M., Daini, O. E., [1]Irivboje, O. A., Odutayo, O. J., [3]Safiyu, K. K., [2]Adebambo, A. O. and Adeyemi, O. A.

Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, P.M.B. 22240, Ogun State, Nigeria.

[1]Agricultural Technology Department, The Federal Polytechnic, Ilaro, P.M.B 50, Ogun State, Nigeria.

[2]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, P.M.B. 22240, Ogun State, Nigeria.

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

***Corresponding author:** sogunleom@funaab.edu.ng

Abstract

The in ovo techniques among other advantages seek to jump start growth and engender early gut development for improved post hatch growth performance of meat birds. In this study the influence of in ovo administration of aqueous extract of oyster mushroom (AEOM) on post-hatch performance of broiler chickens was investigated. A total of 600 hatching eggs of Arbor acre strain of broiler chickens were used for this experiment. Incubation was done at temperature of 37.80°C and relative humidity of 60%. Candling was done on the 14th day of incubation and a total of 336 eggs (56% fertility) were ascertained fertile. These eggs were distributed into three groups containing 112 each for control group, in ovo administration of 0.1 mL AEOM and in ovo administration of 0.2 mL AEOM. In ovo injection of eggs was carried out on the 18th day of incubation. The birds were divided into 4 replicates of 14 birds per replicate and were then brooded for 2 weeks. At the end of the experiment, 2 birds per replicate were randomly selected for carcass evaluation. Data collected were subjected to completely randomized design. Jejunal crypt depth (115.62 µm) and muscular wall thickness (139.23 µm) were higher (P<0.05) in birds from eggs administered 0.1 mL AEOM in ovo at age seven days. The duodenal length of birds from eggs administered 0.1 mL AEOM in ovo was significantly (P<0.05) longer (2.60 cm/100 g) and the ileal muscular wall thickness (248.73 µm) at 56th day though comparable with the values obtained in control. At day 56, intestinal villi histology was improved by in ovo injection of 0.1 mL AEOM. It can be concluded that 0.1 mL AEOM should be administered in ovo for improved intestinal histomorphology of broiler chickens.

Keywords: *in ovo* injection, oyster mushroom, growth performance, carcass characteristics, intestinal morphology and histology, Arbor Acre strain

Influence de l'administration in ovo d'extrait aqueux de pleurote (Pleurotus ostreatus) sur les performances post-éclosion des poulets de chair

Résumé

Les techniques in ovo, entre autres avantages, visent à relancer la croissance et à engendrer un développement précoce de l'intestin pour améliorer les performances de croissance après l'éclosion des oiseaux de chair. Dans cette étude, l'influence de l'administration in ovo d'extrait aqueux de pleurotes (AEAP) sur les performances post-éclosion des poulets de

chair a été étudiée. Un total de 600 œufs à couver de la souche Arbor acre de poulets à griller a été utilisé pour cette expérience. L'incubation a été effectuée à une température de 37,80°C et une humidité relative de 60 %. Le mirage a été effectué le 14e jour d'incubation et un total de 336 œufs (56 % de fertilité) ont été déclarés fertiles. Ces œufs ont été répartis en trois groupes contenant 112 chacun pour le groupe témoin, administration in ovo de 0,1 ml d'AEAP et administration in ovo de 0,2 ml d'AEAP. L'injection in ovo d'œufs a été réalisée au 18ème jour d'incubation. Les oiseaux ont été divisés en 4 répétitions de 14 oiseaux par répétition et ont ensuite été incubés pendant 2 semaines. À la fin de l'expérience, 2 oiseaux par répétition ont été sélectionnés au hasard pour l'évaluation de la carcasse. Les données recueillies ont été soumises à une conception complètement randomisée. La profondeur de la crypte jéjunale (115,62 µm) et l'épaisseur de la paroi musculaire (139,23 µm) étaient plus élevées (P <0,05) chez les oiseaux d'œufs ayant reçu 0,1 mL d'AEAP in ovo à l'âge de sept jours. La longueur duodénale des oiseaux issus d'œufs ayant reçu 0,1 mL de AEAP in ovo était significativement (P < 0,05) plus longue (2,60 cm/100 g) et l'épaisseur de la paroi musculaire iléale (248,73 µm) au 56e jour, bien que comparable aux valeurs obtenues chez le témoin. Au jour 56, l'histologie des villosités intestinales a été améliorée par l'injection in ovo de 0,1 ml d'AEAP. On peut conclure que 0,1 mL d'AEAP devrait être administré in ovo pour améliorer l'histomorphologie intestinale des poulets à griller.

Mots-clés: injection d'in ovo, pleurote, performances de croissance, caractéristiques de la carcasse, morphologie et histologie intestinales, souche Arbor Acre

Introduction

The current commercial hybrid of broiler chickens has great performance which requires quality feed containing high energy content and protein, and good management to enable them maximally express their genetic potentials. To achieve this, several growth promoters including phytogenic materials and systems were used by broiler producers to improve the growth performance of broilers (Sadeghi and Tabiedian, 2005). According to Sklan (2001) the early development of the intestine plays a major role in promoting growth of the newly-hatched chick and post-hatch growth performance of broiler chickens. It was recorded by Sharma and Burmester (1982) that *in ovo* vaccination was first proven to be efficient for Marek's vaccine. This was corroborated by the findings of de Souza (2008) that *in ovo* injection of Marek's vaccine at 18 days of embryonic development resulted into chicks with better protection against virulent Marek disease. Nowadays, *in ovo* injection of eggs has expanded to include injecting the eggs with amino acids (Mehdi *et al.,* 2011) antibiotics (Hadi *et al*., 2014), organic and inorganic salts (Joshua *et al*., 2016; Sogunle *et al*., 2018) to improve the characters of hatching and growth performance of chickens and also to eliminate pathogens and prevention of egg transmission of diseases. Antibiotics are synthetic compounds with antimicrobial activity (Geidam *et al.,* 2009) and are used as growth promotant; to improve health of the birds, feed efficiency, egg production, or as prophylaxis to reduce the incidence of poultry diseases and antimicrobial residues in poultry products in keeping with the 2006 document of the European Union (Ufele and Ogbumuo, 2018). In Nigeria, the indiscriminate use and inappropriate administration of synthetic antibiotics have led to occurrence of harmful residues in edible poultry tissues (Shareef *et al*., 2009) thereby engendering the research in recent years for safe alternatives to synthetic antibiotics including phytobiotics such as garlic, mushrooms and propolis (Galal *et al*., 2008; Sogunle *et al*., 2019). Oyster mushroom (*Pleurotus ostreatus*), was reported to have antioxidant and

immunomodulatory effects (Elmastas *et al*., 2007, Sogunle *et al*., 2019), improve growth, immunity (Ziaran *et al.,* 2005; Khojasteh and Shivazad, 2006) and intestinal health of broiler chickens and layers (Guo *et al*., 2003; Galal *et al*., 2008; Giannenas *et al*., 2010). However, there is paucity of information on the influence of *in ovo* administration of phytobiotics such as aqueous extract of oyster mushroom in poultry production. This study was thereby carried out to determine the post-hatch performance of broiler chickens on *in ovo* administration of aqueous extract of oyster mushroom.

Materials and methods

Experimental site and management of eggs

The experiment was carried out at the Poultry unit, Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The hatchery experiment was carried out in the hatchery of the College of Animal Science and Livestock Production, FUNAAB. A total of 600 Arbor acre broiler chicken hatching eggs were purchased from a reputable breeder farm in Ibadan, Oyo state. The hatching eggs were sorted and balanced for weight. Thereafter, the hatching eggs and incubator were fumigated using $KMnO_4$ and formalin in ratio 2 to 1 for 20 minutes in an enclosed chamber before setting. Incubation was done at temperature of 37.80°C and relative humidity of 60%. Candling was done on the 14^{th} day.

After candling the 600 eggs, a total of 336 eggs (56% fertility) were ascertained to contain viable embryos. These eggs were distributed into three groups containing 112 (control group), 112 (*in ovo* administration of 0.1 ml AEOM) and 112 (*in ovo* administration of 0.2 ml AEOM).

Source and preparation of aqueous extract of oyster mushroom

Fresh oyster mushrooms (*Pleurotus ostreatus*) were purchased from Ibadan, Oyo State. The mushrooms were properly rinsed so as 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 20 minutes. The newly formed extracts were then cooled and strain-off the mushrooms with the aid of a sieve. Thereafter, 20% oyster mushroom extract (20 mL of oyster mushroom extract into 80 ml of deionised water). The newly formed solution 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 (Sogunle *et al.,* 2019).

Procedure for in ovo injection aqueous extract of oyster mushroom

On the 18^{th} day of embryonic development, the eggs were injected with the 20% aqueous extract of oyster mushroom (after sterilization (132ºC for 4 minutes to prevent contamination) using 24-gauge hypodermic needle (Bhanja *et al*., 2004). The surface of the broad end of the eggs was sterilized with 30% ethanol. After *in ovo* injection, the injected sites (via the air cell into the amniotic fluid) on the eggs were sealed with candle wax and the eggs were transferred to the hatching compartment. Hatchability was determined as a percentage of hatched chicks to the fertile eggs.

Experimental animals and management

A total of 66 chicks (58.93% hatchability) were hatched from the control group, 59 chicks (52.68% hatchability) hatched from the 0.1 mL *in ovo* administration group and 56 chicks (50.00% hatchability) hatched from the 0.2 mL *in ovo* administration group. After hatching, the chicks were brooded for two weeks and the experiment lasted for six weeks. The birds were further

distributed into four replicates of 14 birds per replicate. Water and feed were supplied *adlibitum*, and medication and vaccination schedule was adhered to. Feed contained 3200 ME Kcal/kg and 22.76% CP at the starter phase, and 3000 ME Kcal/kg and 20.26% CP at finisher phase.

Determination of growth performance

Data were taken weekly on the growth performance of the bird: feed intake and weight gain. The feed conversion ratio, total feed intake, daily feed intake per bird and survivability were calculated.

Carcass characteristics determination

At the end of the 8th week, two birds per replicate were selected for carcass characteristics. The birds were slaughtered and dressed following conventional procedure by severing the carotid artery and jugular vein. The birds were bled completely and the skin was removed. The live weight and dressed weight were recorded.

Dressing percentage (%) =

$$\frac{\text{Dressing Weight (g)}}{\text{Live weight (g)}} \times 100$$

Organs which included gizzard, liver, spleen, heart, bursa, proventriculus and the intestines (duodenum, jejunum and ileum) were weighed. Cut-up parts of the birds such as the breast, back, thighs and drumsticks were also weighed and expressed as percentages of the live weight.

Cut-up part percentage (%) =

$$\frac{\text{Cut part Weight (g)}}{\text{Live Weight (g)}} \times 100$$

Intestinal morphometry and histology examination

At 7th and 56th days of the experiment, two birds per replicate were slaughtered, skinned to remove segments of the small intestine. Intestinal samples from the three treatments were dehydrated, cleared, and embedded in 10% formalin (100 mL formalin, 900 mL distilled water). Segments (1 cm) were removed (cut) from the tip of the duodenum, jejunum (separated from the ileum at the Meckel diverticulum) and ileum (separated at the ileo-caecal/caeco-colic junction).

Morphometric indices such as the weight, length of each of the sections of the small intestine were measured. The weight was determined using sensitive scale (0.01 g) and expressed as a percentage of the live weight. The length was measured using a metre rule and expressed in cm/100 g live weight.

Statistical analysis

Data collected were subjected to Analysis of Variance (ANOVA) in a completely randomized design. Significantly ($P<0.05$) different means among variables were separated using Tukey test as contained in Minitab® Version 17.1.0 (Minitab, 2013).

$Yij = \mu + Ai + Eijk$

Where:

Yij = Individual Observation

μ = Overall Mean

Ai = Effect of Factor A (*in ovo* injection of aqueous extract of oyster mushroom)

*E*ijk = Experimental error

Results and discussion

Effect of in ovo administration of aqueous extract of oyster mushroom on growth performance of broiler chickens

The results obtained on the influence of *in ovo* administration of aqueous extract ofoyster mushroom on growth performance indices of broiler chickens are shown in Table 1. The results at starter phase corroborated the findings of Lopes *et al*. (2006) and Leitão*et al*. (2006) who reported no significant difference at 10 days of age and at 16 days of age when nutrients are inoculated *in ovo*. However, it contradicted the findings of Salmanzadeh (2012) who reported significant difference in the growth performance of chicks at starter 21 days of age. Also, there was no significant

effect of *in ovo* administration of AEOM on growth performance of the broiler chickens at finisher phase. This result was in line with the findings of Lopes *et al.* (2006) who found no significant difference in the body weight gain of 46 days old broiler chicken fed nutrient *in ovo*. Also, result is consistent with the results of Gore and Qureshi (1997) who found no significant effect at hatch, 35 days of age and finisher phase when nutrient is administered to broiler chickens *in ovo*. On the contrary, Sogunle *et al*. (2018) reported significant difference in the weight gain of broiler chickens on *in ovo* injection of inorganic salts of Zn, Cu and Se. The results of the present study also contrasted with the findings of Giannenas *et al*. (2010) who reported that feed containing oyster mushroom increased body weight gain and enhance feed intake in broiler chickens. The difference in findings could be attributed to the differences in the nutrients used *in ovo* and the route of administration.

Table 1: Effect of varying levels of *in ovo* administration of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) on the growth performance of broiler chickens

	Levels of *in ovo* administration of AEOM				
Parameter	**Control**	**0.1 mL *in ovo***	**0.2 mL *in ovo***	**SEM**	**P-Value**
0 to 28 days					
Initial weight (g/bird)	33.69	33.71	32.96	0.23	0.100
Final weight (g/bird)	464.73	429.37	481.25	17.46	0.509
Daily weight gain (g/bird)	15.39	14.13	16.010	0.62	0.489
Daily feed intake (g/bird)	28.52	28.04	28.64	0.65	0.936
Feed conversion ratio	1.86	2.03	1.79	0.06	0.362
29 to 56 Days					
Initial weight (g/bird)	464.73	429.37	481.25	17.46	0.509
Final weight (g/bird)	1736.75	1579.25	1765.75	65.50	0.501
Daily weight gain (g/bird)	45.42	41.06	45.87	2.13	0.641
Daily feed intake (g/bird)	100.92	116.19	111.88	4.24	0.348
Feed conversion ratio	2.29	2.87	2.43	0.13	0.209
Survivability %	79.46	90.83	91.66	3.59	0.332

SEM = Standard Error of Means
AEOM = Aqueous extract of oyster mushroom

Effect of in ovo administration of aqueous extract of oyster mushroom (AEOM) on the carcass characteristics of broiler chickens

Table 2 shows the results of the influence of AEOM on the carcass characteristics of broiler chickens. The non-significant results on growth performance translated into the non-significant results on carcass performance. The results corroborate with the findings of Al-Daraji *et al*. (2012) who found no significant difference in the carcass characteristics and organ weight of chickens administered nutrients *in ovo*.

Effect of varying levels of in ovo administration of aqueous extract of oyster mushroom (AEOM) on intestinal development and morphometry of broiler chickens at 7 days of age

In Table 3, the effects of varying levels of *in ovo* administration of AEOM on intestinal development and morphometry of broiler chickens at seven days of age are shown. Intestinal development and maturation are known to play a major role in promoting growth of the newly- hatched chick and enhance post-hatch growth performance of broiler chickens (Dibner, 1999; Uni *et al*., 1999). Also, significant increase in the intestinal development results in improved performance of poultry (Cheled-Shoval *et al.,* 2011). The non-significant result in the

intestinal development at early stage contradicted the report by Uni *et al*. (1999) who found rapid increase in the intestinal development of pullets when administered nutrient *in ovo*. This shows that the dosage used and concentration of AEOM administered *in ovo* was adequate to have influenced morphometry of the intestine of broiler chickens at day seven of age. However, results on the morphometry of the duodenum, jejunum and ileum in broiler chickens at starter phase shows significant differences in the jejunal villus height, crypt depth and muscular wall thickness at day seven of age. This results corroborated the findings of Soltani *et al*. (2019) who found significant increase in the jejunal villi height of broiler chickens when administered phytobiotics *in ovo*.

Effect of varying levels of in ovo administration of aqueous extract of oyster mushroom (AEOM) on intestinal development and morphometry of broiler chickens at 56 days of age

Table 4 shows the influence of varying levels of *in ovo* administration of AEOM on intestinal development and morphometry of broiler chickens at 56 days of age**.** Oyster mushroom which contains vitamins such as: thiamin, riboflavin, niacin and folic acid (Sadler, 2003) significantly increased the duodenal length of broiler chickens at finisher phase. This result is in line with the findings of Bohorquez *et al*. (2011) who recorded significant increase in the length of the duodenum at finisher phase when administered vitamins *in ovo.* Soltani *et al*. (2019) also reported significant increase in duodenal parameters of broiler chickens when administered organic substance *in ovo*. Results of the morphometry of colon length showed significantly highest muscular wall thickness in birds from eggs on *in ovo* administration of AEOM up to 0.2 ml and this is comparable to the value obtained in birds on the control. These results supported the findings of Bohorquez *et al*. (2011) who reported that administration of vitamin *in ovo* improves the secretion of intestinal mucus and speeds up maturation of villus epithelial. The improved intestinal morphometric indices in birds on AEOM could be due to the presence of vitamins B and C in oyster mushroom.

Table 2: Effect of varying levels of *in ovo* administration of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) on carcass characteristics of broiler chickens at the 56th day

	Levels of *in ovo* administration of AEOM				
Parameters	**Control**	**0.1 mL *in ovo***	**0.2 mL *in ovo***	**SEM**	**P-Value**
Carcass Characteristics					
Live weight, g	1712.50	1537.50	1712.50	49.79	0.275
Dressing, %	62.02	64.89	63.60	0.81	0.390
Cut-up Parts					
Thigh, %	9.29	9.65	9.59	0.31	0.903
Drumstick,%	8.97	8.59	8.80	0.28	0.881
Back, %	12.65	12.98	12.04	0.31	0.507
Breast, %	14.71	14.29	15.05	0.35	0.723
Organs					
Heart, %	0.47	0.55	0.52	0.02	0.560
Proventriculus, %	0.74	0.59	0.48	0.09	0.587
Gizzard, %	1.88	1.95	1.80	0.07	0.737
Liver, %	1.93	2.48	2.31	0.19	0.565
Pancreas, %	0.28	0.36	0.36	0.02	0.255
Lymphoid organs					
Thymus, %	0.37	0.22	0.36	0.03	0.183
Bursa, %	0.19	0.13	0.06	0.03	0.409
Spleen, %	0.14	0.16	0.13	0.01	0.673

SEM = Standard Error of Mean
AEOM = Aqueous extract of oyster mushroom

Table 3: Effect of varying levels of *in ovo* administration of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) on intestinal development and morphometry of broiler chickens at 7 days of age

	Levels of *in ovo* administration of AEOM				
Parameters	**Control**	**0.1 mL *in ovo***	**0.2 mL *in ovo***	**SEM**	**P-Value**
Live Weight, g	53.50	57.50	52.50	3.29	0.87
Intestinal Development					
Duodenum, cm/100g	22.17	22.31	22.71	0.61	0.95
Jejunum, cm/100g	48.60	44.83	50.02	2.26	0.73
Ileum, cm/100g	42.56	46.48	50.60	3.56	0.75
Caeca, cm/100g	18.03	15.85	16.82	0.96	0.75
Colon, cm/100g	5.88	3.91	5.18	0.67	0.59
Duodenum morphometry					
Villus Height, µm	384.56	342.02	261.56	54.24	0.93
Villus Width, µm	40.82	63.00	50.75	6.26	0.47
Crypt Depth, µm	39.93	63.79	51.84	7.59	0.50
Muscular Wall Thickness, µm	78.04	93.31	91.66	11.16	0.73
Jejunum morphometry					
Villus Height, µm	290.62^{c}	351.57^{b}	415.60^{a}	37.39	0.01
Villus Width, µm	45.65	68.73	59.16	7.25	0.51
Crypt Depth, µm	..	115.62^{a}	57.92^{b}	13.83	0.01
Muscular Wall Thickness, µm	53.94^{b}	139.23^{a}	68.61^{b}	17.62	0.04
Ileum morphometry					
Villus Height, µm	581.44	381.45	522.43	43.04	0.52
Villus Width, µm	68.35	70.38	83.60	4.00	0.48
Crypt Depth, µm	71.10	83.04	115.82	9.91	0.45
Muscular Wall Thickness µm	111.97	137.23	146.77	8.81	0.46

$^{a, b}$ Means in the same row with different superscripts differ significantly (P<0.05); SEM = Standard Error of Means.
AEOM = Aqueous extract of oyster mushroom

Table 4: Effect of varying levels of *in ovo* administration of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) on intestinal development and morphometry of broiler chickens at 56 days of age

	Levels of *in ovo* administration of AEOM				
Parameters	**Control**	**0.1 mL *in ovo***	**0.2 mL *in ovo***	**SEM**	**P-Value**
Live Weight, g	1545.5	1690.0	1840.0	210	0.65
Intestinal Development					
Duodenum, %	1.24	1.24	0.99	0.10	0.29
Duodenum, cm/100g	2.35^{ab}	2.60^{a}	1.72^{b}	0.13	0.03
Jejunum, %	3.010	3.00	2.74	0.31	0.80
Jejunum, cm/100g	5.782	5.58	4.68	0.46	0.33
Ileum, %	2.58	2.69	2.66	0.56	0.99
Ileum, cm/100g	6.23	5.67	5.24	0.24	0.13
Caeca, %	0.74	0.70	0.63	0.05	0.45
Caeca, cm/100g	2.73	2.30	2.20	0.23	0.36
Colon, %	0.13	0.10	0.31	0.06	0.22
Colon, cm/100g	0.61^{a}	0.41^{b}	0.61^{a}	0.01	0.02
Duodenum morphometry					
Villus Height, µm	1294.09	1233.95	1470.90	104	0.37
Villus Width, µm	112.879	100.79	84.97	19.3	0.63
Crypt Depth, µm	137.98	162.50	112.87	18.8	0.31
Muscular Wall Thickness, µm	175.24	186.26	157.31	30.4	0.80
Jejunum morphometry					
Villus Height, µm	914.86	1112.52	936.98	189	0.74
Villus Width, µm	113.79	233.37	87.31	72.6	0.42
Crypt Depth, µm	103.68	144.05	68.75	28.1	0.30
Muscular Wall Thickness µm	244.79	167.76	149.31	35.40	0.28
Ileum morphometry					
Villus Height, µm	987.01	649.53	1092.39	188	0.35
Villus Width, µm	123.45	92.74	106.34	15.2	0.45
Crypt Depth, µm	146.71	133.70	113.18	30.7	0.75
Muscular Wall Thickness µm	228.17^{ab}	248.73^{a}	158.69^{b}	12.2	0.02

^{ab} Means in the same row with different superscripts differ significantly (P<0.05); SEM = Standard Error of Mean.
AEOM = Aqueous extract of oyster mushroom

Conclusion and recommendation

It can be concluded that administration of 0.1 mL aqueous extract of oyster mushroom *in ovo* improved the intestinal development of broiler chickens. Therefore, administering oyster mushroom *in ovo* at 0.1 mL can be beneficial to intestinal health and subsequent overall development of broiler chickens.

References

Aderibigbe, O. B., Sogunle, O. M., Egbeyale, L. T., Abiola, S. S., Ladokun, O. A.and Ajayi, O. L.2013. Pelletized Feed of Different Particle Sizes: Effects on Performance, Carcass Characteristics and Intestinal Morphology of Two Strains of Broiler Chicken. Pertanika Journal of Tropical Agricultural Science, 36 (2): 128 – 144.

Al-Daraji, H. J., Al-Mashadani, A. A., Al-Hayani, W. K., Al-Hassani, A. S. and 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 (No. 2).

Bhanja, S. K., Mandal, A. B., and 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*, 39: 212-218.

Bohorquez, D. V., Bohorquez, N. E. and Ferket, P. R. 2011. Ultrastructural development of the small intestinal mucosa in the embryo and turkey: A light and electron microscopy study. *Poultry Science,* 90: 842-855.

Cheled-Shoval, S.L., Amit-Romach, E., Barbakov, M. and Uni, Z. 2011.The effect of *in ovo* administration of mannan oligosaccharide on small intestine development during the pre- and post-hatch periods in chickens. *Poultry Science*, 90: 2301-2310

Cheung, P. C. K. 2010. 'The nutritional and health benefits of mushrooms'. *Nutrition Bulletin*, 35: 292-299.

de Souza, F. M. 2008. Basic aspects of *in ovo* injection in commercial hatcheries. *Hatchery expertise online. Issue No*. 20 / September 2008.

Dibner, J. 1999. Feeding hatchling poultry. Avoid any delay. *Feed International,* December, 30–34.

Elmastas, M., Isildak, O., Turkekul, I. and Temur, N. 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis* 20: 337–345.

Galal , A., Abd el – Motaal, A. M., Ahmed, A. M. H. and Zaki, T. G. 2008. Productive performance and immune response of laying hens as affected by dietary propolis supplementation. International Journal of Poultry Science, 7: 272 – 278.

Geidam, Y. A., Usman, H., Musa, H. I., Anosike, F. and Adeyemi, Y. 2009. Oxytetracycline and Procain Penicillin residues in tissues of slaughtered cattle in Maiduguri, Borno state, Nigeria. *Terrestrial Environment Toxicolology*, 3 (2): 68 - 70.

Giannenas, I., Pappas, I. S., Mavridis, S., Kontopidis, G., Skoufos, J. and Kyriazakis, I. 2010. Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet. *Poultry Science,* 89(2): 303–311.

Gore, A.B. and Qureshi, M. A. 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. *Poultry Science*, 76: 984–991.

Guo, F. C., Kwakkel, R. P., Williams, B. A., Li, W. K., Li, H. S., Luo, J. Y., Li, X. P., Wei, Y. X., Yan, Z. T and Verstegen, M. W. A. 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on growth performance of broilers. *British Poultry Science*, 45: 684–689.

Hadi, S. M., Kadhim M. I. and Shatha, I. Y. 2014. Effect of shock and elevated levels of mannitol on callus growth, regeneration and proline accumulation in *Ruta graveolens* cultures. *International Journal of Current Microbiology and Applied Sciences*, 3 (11): 2319-7706.

Joshua, P.P., Valli, C. and Balakrishnan, V. 2016. Effects of *in ovo* supplementation of Nano form of Zinc, Copper and Selenium on post-hatch performance of broiler chicken. *Veterinary World*, 9 (3):287–294.

Khojasteh Shalmany, S. and Shivazad, M. 2006. The effect of diet propolis supplementation on Ross broiler chicks' performance. *International Journal of Poultry Science*, 5: 84–88.

Leitão, R. A., Leandro, A. A., Pedroso, N.S.M., Stringhini, J. H., Oliveira Neto, J. R. and Café, M. B. 2006.Effect of glucose in ovo supplementation at starter performance of broilers.*Brazilian Journal Avian Science Camp*, 7:69

Lopes, K.L.A.M., Pedroso, A. A. Leandro, N.S.M., Stringhini, J. H. and Barbosa, C. E. 2006. Glutamine *in ovo* inoculation effect at the starter performance of broilers. *Brazilian Journal Avian Science Camp*, 8:103

Manzi, P and Pizzoferrato, L. 2000. 'Beta-glucans in edible mushrooms'. *Food Chemistry*, 68: 315-318.

Mehdi, S., Yahya, E., Habib, A. S. and 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,* 1(4):164-168.

Minitab 17 Statistical Software. 2013. Stable release 17.1.0.

Sadeghi, G. H and Tabiedian. S. A. 2005. Effect of Different Energy to Protein Ratio and Tallow Supplementation on Broiler Performance. *International Journal of Poultry Science,* 4 (12): 976-981.

Sadler, M. 2003. Nutritional properties of edible fungi. British Nutritional Found Nutrients Bulletin, 28: 305-308.

Salmanzadeh, M. 2012. The Effects of *in ovo* injection of glucose on hatchability, hatching weight and subsequent performance of newly-hatched Chicks. *Brazilian Journal of Poultry Science,* 14 (2): 71-158.

Shareef, A. M., Jamel, Z. T. and Yonis, K. M. 2009. Detection of antibiotic residues in stored poultry products. *Iraq Journal of Veterinary Science*, 23(1): 45-48.

Sharma, J. M. and Burmester, B. R. 1982. Resistance of Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. *Avian Disease*. 27:134–149.

Sklan, D. 2001. Development of the digestive tract of poultry. *Poultry Science*, 57:415–428.

Sogunle, O. M., Elangovan, A. V., David, C. G., Ghosh, J. and Awachat, V.

B. 2018. Response of broiler chickens to *in ovo* administration of inorganic salts of zinc, selenium and copper, and their combination. *Slovak Journal of Animal Science*, 51 (1): 9-18.

Sogunle, O. M., Labinjo, O. S., Olanite, J. A. and 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,* 68 (261): 24-30.

Soltani, T., Salarmoini, M., Afsharmanesh, M. and Tasharrofi, S. 2019. The Effects of *in ovo* Injection of Ascorbic Acid on Hatchability, Growth Performance, Intestinal Morphology, and tibia breaking strength in 36h post-hatch fasted broiler chickens. *Poultry Science Journal*, 7(1): 43-49.

Subramaniyan, S. A., Park. J. R., Siddiqui, R. R., Yoo, D. J. and Shim, K. S. 2019.E*ff*ect of *in ovo* injection of L-arginine in di*ff*erent chicken embryonic development stages on post-hatchability, immune response, and myo-D and myogenin proteins. *Animal Science Journal*, 9: 357-377.

Ufele, A. N. and Ogbumuo, P. N. 2018. Effect of pearl millet (*Pennisetum glaucum* L. Br.) and garlic meal (*Allium sativum* L.) on growth performance of broiler chicks. *American Journal of Zoological Research*, 6 (1): 8-11.

Uni, Z., Ganot, S. and Sklan, D. 1998. Post-hatch development of mucosal function in the broiler small intestine. *Poultry Science*. 77:75–82.

Uni, Z., Noy, Y. and Sklan, D. 1999. Post-hatch development of small intestinal function in the poult. *Poultry Science*, 78: 215–222.

Ziaran, H. R., Rahmani, H. R. and Pourreza, J. 2005. Effect of dietary oil extract of propolis on immune response and broiler performance. *Pakistan Journal of Biological Science*, 8:1485–1490.

Received: 8th September, 2021
Accepted: 28th January, 2022