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# Journal of World's Poultry Research

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Effects of Diet Containing Fermented Canola Meal on Performance, Blood Parameters and Gut Health of Broilers chickens

## Research Paper

### Effects of Diet Containing Fermented Canola Meal on Performance, Blood Parameters and Gut Health of Broiler Chickens.

Elbaz AM.

*J. World Poult. Res.* 11(1): 01-07, 2021; pii: S2322455X2100001-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.1>

**ABSTRACT:** The current research aimed to study the effects of the fermented canola meal (*Lactobacillus*) diet on productive performance, blood parameters, and gut health of broiler chickens under high ambient temperature conditions. A total number of 320 (Ross-308) one-day-old broiler chickens were allocated randomly into four experimental groups for 42 days. Four experimental groups with four types of diet, including the control group (CON) received basal diet, and three other experimental groups were supplemented with 20% of the canola meal (CM), 20% fermented canola meal (FCM), and 20% canola meal with probiotic (PCM). The chickens that fed FCM presented improvement in live body weight, feed conversion ratio, and higher nutrient digestibility, compared to CM and PCM groups. Serum glucose, total protein, albumin, and aspartate aminotransferase (AST) of levels of chickens fed by FCM were higher than chickens fed CM and PCM, while there was a decrease in cholesterol. Fermented canola meal resulted in some noticeable beneficial changes in the cecum microflora communities through increasing the population of *Lactobacillus* spp. and decreasing the *Escherichia coli* and improved its morphology by increasing villus height. The results indicated that the fermentation of canola meal has enhanced performance, nutrient digestibility, and gut health, which allow using greater amounts of fermented canola meal as a replacement of soybeans meal in the broiler diet.

**Keywords:** Broiler, Canola meal, Fermentation, Gut health, Performance, Serum parameter.

[Full text-PDF] [XML] [Crossref Metadata]

Sani NA, Ugochukwu CI, Abalaka SE, Saleh A, Muhammed MS, Oladele SB, Abdu PA, and Njoku C (2021). Clinicopathological Findings in Suspected Cases of Virus-induced Neoplastic Diseases in Commercial Layer Chickens in Nigeria. *J. World Poult. Res.*, 11 (1): 08-15. DOI: <https://dx.doi.org/10.36380/jwpr.2021.2>

### Clinicopathological Findings in Suspected Cases of Virus-induced Neoplastic Diseases in Commercial Layer Chickens in Nigeria.

Sani NA, Ugochukwu CI, Abalaka SE, Saleh A, Muhammed MS, Oladele SB, Abdu PA, and Njoku C.

*J. World Poult. Res.* 11(1): 08-15, 2021; pii: S2322455X2100002-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.2>

**ABSTRACT:** Avian neoplastic diseases, including Marek's disease (MD), avian leukosis (AL), and reticuloendotheliosis (RE), are of economic importance in the chicken industry. However, it is difficult to differentiate MD from AL and RE by clinical signs and postmortem examination. Therefore, the present study aimed to classify the avian neoplastic diseases affecting commercial layer chickens in Nigeria using clinical history, postmortem examination, and histopathological technique. Carcasses of commercial layer chickens from 7 and 20 poultry farms in Kaduna and Plateau States were studied, respectively, from February 2017 to March 2018. The age, morbidity, and mortality rates in each of the affected farms were determined. Detailed postmortem examinations were carried out on the carcasses from the affected farms, and organs observed to have neoplastic lesions were fixed in 10% neutral buffered formalin for histopathology. The age means of the affected layers were 20.6 weeks and 20.8 weeks in Kaduna and Plateau States, respectively. The average morbidity rates of neoplasm in the affected layers were 3.9% and 9.3% in Kaduna and Plateau States, respectively, while the average mortality rates were 8.6% and 8.5% in Kaduna and Plateau States, respectively. The clinical observation of affected chickens indicated that they were anorexic and emaciated. Generally, the neoplastic lesions were characterized by white to gray, multifocal, firm nodules of varying sizes on the affected organs. In Kaduna State, the neoplasms were commonly observed on the liver (85.7%), spleen (71.4%), heart (42.9%), and kidneys (42.9%), while in Plateau State, the affected organs included liver (50%), spleen (25%), proventriculus (25%) and lungs (25%). The histopathological changes in the affected tissues were similar and characterized predominantly by the infiltration of lymphocytes, lymphoblasts, and macrophages. The patterns of distribution of the pleomorphic neoplastic cells within the liver were multifocal and perivascular in most cases. Findings from the current study indicated that cases of neoplasms in commercial layer chickens in Kaduna and Plateau States, Nigeria, could be attributed to MD.

**Keywords:** Avian neoplastic diseases, Layer chickens, Pathology.

[Full text-PDF] [XML] [Crossref Metadata]



## Effect of Pre-Slaughter Antacid Supplementation of Drinking Water on Carcass Yield and Meat Quality of Broiler Chickens.

Namted S, Srisuwan K, Bunchasak C, and Rakangthong C.

*J. World Poult. Res.* 11(1): 16-21, 2021; pii: S2322455X2100003-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.3>

**ABSTRACT:** Antacid is a mixture of sodium bicarbonate, bicarbonate, and citric acid, which can neutralize stomach acidity and may stabilize the pH of post-mortem carcass and meat. Therefore, the present study aimed to investigate the carcass and meat quality of broiler chickens by supplementing the antacid in drinking water. A total of 48 male broiler chickens (Ross 308) were divided into two groups that the first group was the control group (did not receive antacid supplementation in the drinking water) and the second group was supplemented with antacid in drinking water (0.10%) for three days pre-slaughter. It was found that the antacid supplementation increased the percentage of breast meat, while carcass yield, and thigh, drumstick, and wing were not significantly affected. The pH of breast meat 45 minutes and the drip loss at 24 hours post-slaughter was significantly higher. The shear-force of breast meat was reduced ( $P < 0.05$ ) by antacid supplementation. For the color of the breast meat, there were no significant differences in lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) between the two groups, but the total difference in the color of meat was slightly increased. It can be concluded that supplementing the drinking water with an antacid for three days before slaughter improves the carcass and meat quality of broiler chickens by maintaining the pH and water holding capacity of the meat.

**Key words:** Antacid, Broiler chickens, Carcass yield, Meat quality.

[Full text-PDF] [XML] [Crossref Metadata]



## Effects of Acetaminophen and Vitamin Supplement on Feed Intake, Body Weight, and Acute Pain Responses of Pullets Subjected to Beak-trimming.

Okoroafor ON, Okereke HN, and Udegbumam RI.

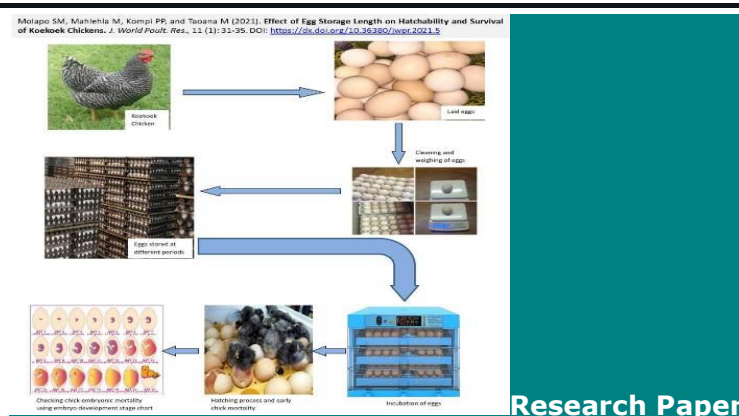
*J. World Poultry Res.* 11(1): 22-30, 2021; pii: S2322455X2100004-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.4>

**ABSTRACT:** The first experiment aimed to compare the effects of manual and electric beak-trimming on feed intake, body weight, and some biochemical parameters of eight-week-old pullets. A total of 20 pullets aged 3 weeks were assigned to two treatment groups; those in group A were manually debeaked while the samples in group B were electro debeaked. The findings indicated that 2 hours post-beak-trimming (PBT), the packed-cell volume of group A was significantly higher than that of group B. Plasma cortisol level of group A was significantly higher than that of group B at 2 and 6 hours PBT while total plasma protein level of group A was significantly lower than that of the group at 2 and 72 hours PBT. Furthermore, feed intake and body weight markedly decreased in the pullets debeaked with both methods until 72 hours PBT. The chickens' beak trimmed with both methods experienced intense pain of varying degrees lasting up to 72 hours. In the second experiment, 40 eight-week-old pullets were assigned to four groups; group A was the control, group B was treated with a vitamin supplement, group C was treated with acetaminophen, and group D was treated with vitamin supplement plus acetaminophen. After 24 hours, chickens were beak-trimmed using a manual cutter. The results revealed that 2 hours PBT, plasma cortisol level in groups B, C, and D were significantly lower than that of group A. Blood glucose was lowest in groups A and D at 6 and 24 hours PBT, respectively. It is concluded that the pre-treatment with vitamins and NSAIDs could reduce stress and pain in debeaked chickens.

**Keywords:** Anti-stress, Debeaking, Pain, Pullet.

[Full text-PDF] [XML] [Crossref Metadata]



## Effect of Egg Storage Length on Hatchability and Survival of Koekoek Chickens.

Molapo SM, Mahlela M, Kompf PP, and Taoana M.

*J. World Poultry Res.* 11(1): 31-35, 2021; pii: S2322455X2100005-11

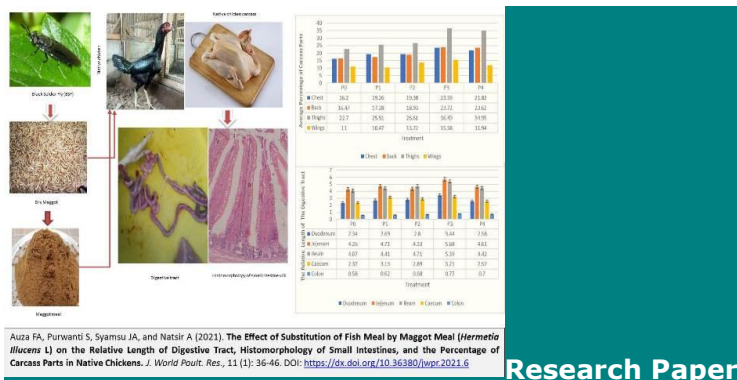
DOI: <https://dx.doi.org/10.36380/jwpr.2021.5>

**ABSTRACT:** Chicken production plays a major in the livelihood of rural people due to the provision of eggs and meat which are high sources of protein. This calls for sustainable production of chickens through strategies aimed at improving the hatchability of eggs and survival of chickens. Therefore, the present study was conducted to determine the effect of egg storage length on egg hatchability and survival of the Koekoek chickens. A total number of 270 eggs were divided into three

treatment groups, and the eggs of each group were stored for 3, 7, and 11 days before incubation. Each treatment consisted of three replicates. The General Linear Model procedure was used to analyze the data. The eggs that were stored for three days before incubation had a higher hatching percentage, compared to those that were stored for 7 and 11 days before incubation. Storing eggs for few days before incubation resulted in reduced embryonic mortality rate and lower mortality of chickens during the first seven days after hatching. Based on these results, is recommended that Koekoek chicken eggs should be stored for three days before incubation to maximize hatchability and survival of chickens before the age of seven days.

**Keywords:** Eggs, Storage, Embryo mortality, Hatchability, Koekoek chicken.

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## The Effect of Substitution of Fish Meal by Maggot Meal (*Hermetia Illucens* L) on the Relative Length of Digestive Tract, Histomorphology of Small Intestines, and the Percentage of Carcass Parts in Native Chickens.

Auza FA, Purwanti S, Syamsu JA, and Natsir A.

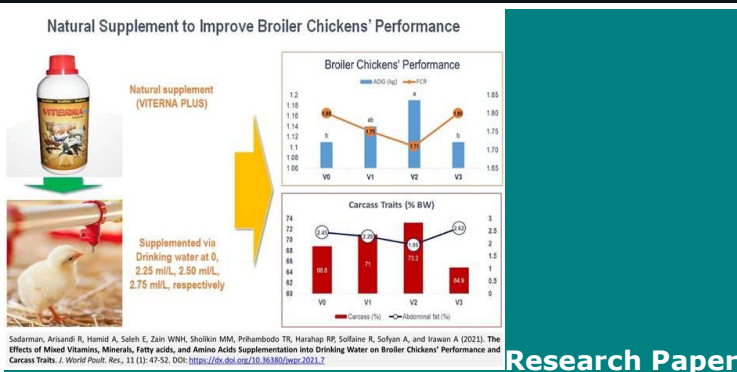
*J. World Poult. Res.* 11(1): 36-46, 2021; pii: S2322455X2100006-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.6>

**ABSTRACT:** The development of the digestive tract organs is closely related to the increased body weight growth in chickens. The present study aimed to determine the effect of using maggot meal as an antibacterial and protein source of fish meal substitution in diets on the relative length of the digestive tract organs, small intestine histomorphology, and the percentage of the native chicken carcass. A total of 140 one-day-old chickens were randomly assigned to one of the five treatments according to a completely randomized design with four replications for each treatment. The treatments included P0 (basal diet + 15% fish meal + 0% maggot meal), P1(basal diet + 11.25% fish meal + 3.75% maggot meal), P2 (basal diet + 7.5% fish meal + 7.5% maggot meal), P3(basal diet + 3.75% fish meal + 11.25% maggot meal), and P4 (basal diet + 0% fish meal + 15% maggot meal). The results showed that the use of maggot meal in P3 had a significant effect ( $P < 0.05$ ) on the relative length, villi height, depth of duodenal crypt, jejunum and ileum, villi surface area, the density of jejunum and ileum villi, and percentage of thigh and wing weight. Besides, the treatment tended to increase the relative length of the caecum and colon, surface area of the duodenal villi, and chest weight percentage. However, the treatment did not affect the duodenal villi density and percentage of back weight in native chickens. The use of maggot meal up to 11.25% in diets can improve the relative length of intestinal, histomorphology of small intestine's villi, and the percentage of carcass parts of native chickens.

**Keywords:** Carcass parts, Digestive tract, Histomorphology, Maggot meal, Native chicken.

[Full text-PDF] [XML] [Crossref Metadata]



## The Effects of Mixed Vitamins, Minerals, Fatty Acids and Amino Acids Supplementation into Drinking Water on Broiler Chickens' Performance and Carcass Traits.



Sadarman, Arisandi R, Hamid A, Saleh E, Zain WNH, Sholikin MM, Prihambodo TR, Harahap RP, Solfaine R, Sofyan A, and Irawan A.

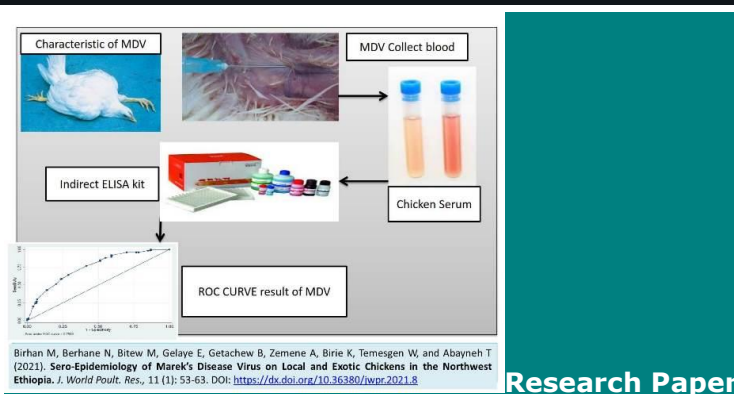
*J. World Poult. Res.* 11(1): 47-52, 2021; pii: S2322455X2100007-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.7>

**ABSTRACT:** The present study was conducted to evaluate the effects of different levels of the feed supplement containing minerals, fatty acids, vitamins, and amino acids added to drinking water on broiler chickens' performance and carcass traits. A total of 100 one-day-old Cobb 707 (mean weight 46.7 g) were randomly assigned into four treatments, including control group (C), C + 2.25 ml/L Viterna Plus (V1), C + 2.50 ml/L Viterna Plus (V2), and C + 2.75 ml/L Viterna Plus (V3). Each treatment group contained 5 replicates of 5 birds in each (25 birds per treatment). Birds were maintained for 28 days. The results suggested that feed supplement at 2.50 ml/L could successfully improve final body weight, performance index, and carcass weight ( $P < 0.05$ ). Concurrently, the treatment also reduced the percentage of abdominal fat ( $P < 0.05$ ). In conclusion, the incorporation of commercial feed supplement containing mixed of minerals, vitamins, and amino acids at 2.50 ml/L into drinking water improved the overall performance of the broiler chickens.

**Keywords:** Broiler chicken, Carcass, Feed supplement, Tropics, Viterna plus.

[Full text-PDF] [XML] [Crossref Metadata]



## Sero-Epidemiology of Marek's Disease Virus on Local and Exotic Chickens in the Northwest Ethiopia.

Birhan M, Berhane N, Bitew M, Gelaye E, Getachew B, Zemene A, Birie K, Temesgen W, and Abayneh T.

*J. World Poult. Res.* 11(1): 53-63, 2021; pii: S2322455X2100008-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.8>

**ABSTRACT:** Marek's disease (MD) is a lymphoproliferative and neuropathic disease of domestic fowl caused by alphaherpesviruses. The current cross-sectional study with a simple random sampling method was undertaken from January 2018 to May 2020. The current study aimed to estimate sero-epidemiology and assess potential risk factors, it is very important to determine MD sero-positivity. Moreover, to measure the association of host and environmental risk factors, the occurrences and spread of MD were identified in local and exotic chickens in Northwest Ethiopia. A total of 768 serum samples from 3 zones were collected and assayed for Marek's Disease Virus (MDV) antibodies using the indirect enzyme-linked immunosorbent assay test. A questionnaire survey was also conducted to gather information on the potential risk factors of MDV sero-positivity, as well as the status of occurrences and spread in the chicken flocks. In the present preliminary study, a high flock and chicken level of MDV was demonstrated, with an overall seroprevalence of 59.11%. The mixed-effect logistic regression analysis of the host potential risk factors showed that the odds of seropositive for MD was significantly higher in local chickens (OR: 1.70, 95% CI: 1.26-2.28) than exotic chickens, higher in chickens getting non-proper feed (OR: 0.26, 95% CI: 0.13-0.54) than getting proper feed, higher in vaccinated chickens (OR: 1.04, 95% CI: 0.76-1.43) than non-vaccinated chickens. Rearing chicken of different batches in one house was decreased the odds of occurrence of MD by 55% (95% CI: 0.38-0.80) than all-in-all-out, higher in the well-ventilated type farms decreased the odds of occurrence of MD by 60% (95% CI: 0.39-0.80) than in poor ones. Litter management when farmers used chickens as fertilizer has decreased the odds of occurrence of MD by 55% (95% CI: 0.01-0.47) and chickens were tossed into open sheds 40% (95% CI: 0.01-0.31), compared to buried chickens management. The study results indicated that the number of farms where farmers wearing no clothes and shoes (95% CI: 0.10-0.58) were significantly decreased the occurrence of MD by 24% than those where farmers were equipped with clothes and shoes. The study area was highest in West Gojjam (OR: 0.40, 95% CI: 0.27-0.58) and South Gondar (OR: 0.19, 95% CI: 0.13-0.28) compared to North Gondar zone. In conclusion, the present study revealed a high flock and chicken seroprevalence level of MDV among chicken flocks in northwest Ethiopia, suggesting that environmental dust/dander and farm management systems might be a source of this disease for chicken infection. Besides, the observed association of MD, sero-positivity with environmental dust/dander, and farm management systems may suggest the economic importance of the disease for chicken production. Therefore, it warrants control attention to reduce its economic and disease spread burden in the study areas. Further works on the



economic impacts, virus isolation, and molecular characterization of the disease are suggested. **Keywords:** Chicken, Marek's Disease, Northwest Ethiopia, Risk factors, Sero-epidemiology.

[Full text-PDF] [XML] [Crossref Metadata]

The thumbnail features a diagram on the left showing 'Poor biosecurity practices' leading to 'Antibiotic resistance' and 'Disease outbreak' (Avian Influenza H5N1). On the right, a table summarizes the study data across three regions: Center, Littoral, and West. The table includes columns for 'Study areas', 'Mean biosecurity scores', 'Number of farms', 'Number of farms with disease outbreak', and 'Farm size'.

Study areas	Mean biosecurity scores	Number of farms	Number of farms with disease outbreak	Farm size
Center	2 (40.0)	6 (40.0)	5 (45.5)	>1000
	3 (60.0)	6 (55.5)	≤1000	
Littoral	2 (53.3)	4 (40.0)	>1000	
	3 (46.7)	6 (60.0)	≤1000	
West	2 (40.0)	4 (33.3)	>1000	
	3 (60.0)	8 (66.7)	≤1000	

Tatfo Keutchatang FDP, Isabelle Sandrine B N, Medoua Nama G, and Kansci G (2021). Biosecurity Practices and Characteristics of Poultry Farms in Three Regions of Cameroon. *J. World Poult. Res.*, 11 (1): 64-72. DOI: <https://dx.doi.org/10.36380/jwpr.2021.9>

## Biosecurity Practices and Characteristics of Poultry Farms in Three Regions of Cameroon.

Tatfo Keutchatang FDP, Bouelet Ntsama IS, Medoua Nama G, and Kansci G.

*J. World Poult. Res.* 11(1): 64-72, 2021; pii: S2322455X2100009-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.9>

**ABSTRACT:** The outbreak of diseases is the main factor affecting poultry production in Cameroon. The implementation of biosecurity measures in poultry farms is essential to reduce disease outbreaks. This study aimed to assess biosecurity practices in poultry farms in three regions of Cameroon. The study was carried out using a structured questionnaire on 90 randomly selected poultry farms. Most of the farmers were men (85%) with deep litter (77.8%), battery cage (2.2%), and both deep litter and battery cage (20.0%) housing systems. Amongst the farms surveyed, 9/30 (30.0%) in the Centre; 8/30 (26.7%) in the Littoral; and 13/30 (43.3%) in the West were aware of biosecurity measures. The biosecurity score (BS) of surveyed farms ranged between 2 and 3. The findings indicated that 39 farms (12 in the Centre, 14 in the Littoral, and 13 in the West) were at moderate risk, and 51 farms (18 in the Centre, 16 in the Littoral, and 17 in the West) were at high risk. Reasons for keeping chickens and the number of chickens per farm did not significantly influence BS, while the farm category could significantly affect it. The outbreak of diseases correlated with BS, showing a tendency of increase in the outbreak of diseases with increasing BS. This study underlines the fact that biosecurity practices in Cameroon have not been well implemented by chicken farmers. This leads to disease outbreaks, and consequently, important economic losses as well as massive use of drugs that may be unsafe for human consumption. Therefore, the effective monitoring of biosecurity in chicken farming should be encouraged by extension of training to the farmers to support the efficient production of chickens by respecting biosecurity that drastically reduces the risk of disease outbreaks and provides good quality chicken products for human consumption.

**Keywords:** Assessment, Biosecurity practices, Biosecurity scores, Cameroon, Poultry farms.

[Full text-PDF] [XML] [Crossref Metadata]

The thumbnail includes a title, authors' names (Iswati Iwati, Natsir MH, Ciptadi G, and Susilawati T), and their affiliations. It features two graphs: a bar chart (Graph 1) showing egg production and a line graph (Graph 2) showing luteinizing hormone concentration over time for different treatment groups. The text below the graphs provides a brief description of the data.

Iswati I, Natsir MH, Ciptadi G, and Susilawati T (2021). Egg Production, Fertility, Hatchability and Luteinizing Hormone Profile of Progesterone Hormone Injected to Arabic Gold Chicken (*Gallus turcicus*). *J. World Poult. Res.*, 11 (1): 73-82. DOI: <https://dx.doi.org/10.36380/jwpr.2021.10>

## Egg Production, Fertility, Hatchability and Luteinizing Hormone Profile of Progesterone Hormone Injected to Arabic Gold Chicken (*Gallus turcicus*).

Iswati I, Natsir MH, Ciptadi G, and Susilawati T.

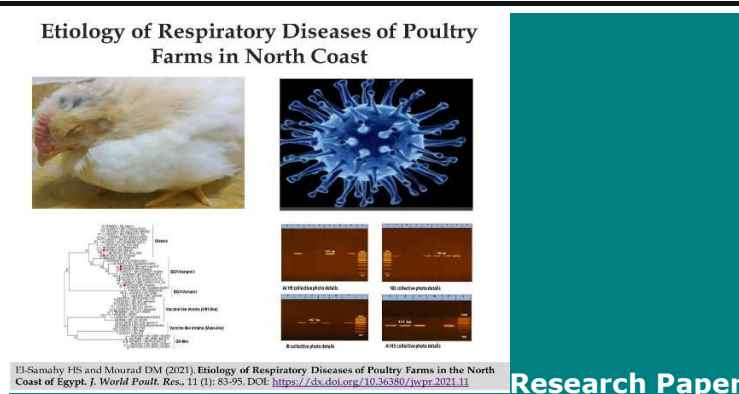
*J. World Poult. Res.* 11(1): 73-82, 2021; pii: S2322455X2100010-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.10>

**ABSTRACT:** The production and reproduction performance of chicken depends on their hormonal status, especially progesterone hormone, which has been known to correlate with egg production. The present study aimed to analyze the effect of progesterone hormone injection on Arabic Gold chickens (*Gallus turcicus*) regarding egg production and luteinizing hormone concentration in blood plasma. A total number of 60 Arabic Gold chickens aged 26-weeks were divided into three groups based on injected hormone concentration (P<sub>0</sub>: control; P<sub>1</sub>: 1 mg/chicken; P<sub>2</sub>: 2 mg/chicken). The study was conducted using a completely randomized design and the obtained data were analyzed with a descriptive analysis for qualitative data and one-way analysis of variance followed with Duncan's Multiple Range Test as a post hoc test for the quantitative data. The results presented that progesterone hormone injection had a significant effect on hen day production two and six weeks after injection. The P<sub>1</sub> group was able to reach its peak production (82.9%) at week 29, while the P<sub>2</sub> group reached its peak at week 26 (78.9%). In addition, it was found that the P<sub>2</sub> group produced a soft-shelled egg and double egg yolk. Progesterone injection led to no significant effect on the egg weight, shape index, fertility, embryo viability, hatchability, and chick weight at hatch. The luteinizing hormone concentration was higher in P<sub>2</sub> (1.52 ng/ml), compared to P<sub>0</sub> (1.36 ng/ml) and P<sub>1</sub> (1.34 ng/ml) groups. It was concluded that progesterone hormone injection during the production phase of Arabic Gold chicken had a significant effect on egg production and caused varying egg production peak and luteinizing hormone concentration.

**Keywords:** Arabic Gold chicken, Egg quality, Hen day production, Luteinizing hormone, Progesterone.

[Full text-PDF] [XML] [Crossref Metadata]



The thumbnail for the research paper features a title 'Etiology of Respiratory Diseases of Poultry Farms in North Coast' at the top. Below the title are several images: a photograph of a white chicken, a 3D model of a virus particle, a phylogenetic tree, and four small panels showing PCR gel electrophoresis results. At the bottom left, there is a citation: 'El-Samahy HS and Mourad DM (2021). Etiology of Respiratory Diseases of Poultry Farms in the North Coast of Egypt. J. World Poult. Res., 11 (1): 83-95. DOI: https://dx.doi.org/10.36380/jwpr.2021.11'. To the right of the thumbnail, the text 'Research Paper' is displayed in a teal box.

## Etiology of Respiratory Diseases of Poultry Farms in the North Coast of Egypt.

El-Samahy HS and Mourad DM.

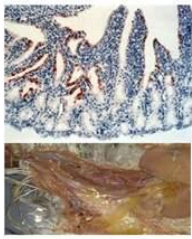
J. World Poult. Res. 11(1): 83-95, 2021; pii: S2322455X2100011-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.11>

**ABSTRACT:** The current study aimed to identify the respiratory problems in poultry farms located in the north coast of Egypt from October 2018 to November 2019. A total of 89 poultry flocks (79 broilers, 5 layers, 3 ducks, and 2 turkeys) were investigated for four major viral respiratory pathogens, namely avian influenza (AI) H9N2, AI H5 subtypes, Newcastle Disease (ND), and Infectious Bronchitis (IB) viruses. All 89 flocks were subjected to real-time PCR to investigate AI H9N2 virus. The samples of 31, 43, and 15 out of 89 flocks were selected for the investigation of ND, IB, and AI H5 subtypes viruses, respectively, using real-time PCR. Sample selection was performed according to the mortalities, clinical signs, and post mortem lesions. The positive findings indicated that 22 out of 89 flocks were positive for AI H9N2 virus (2 layers + 20 broilers), 32 out of 43 flocks were positive for IB virus (2 layers + 30 broilers), 24 out of 31 flocks were positive for ND virus (1 Duck + 1 layer+ 22 broilers) and 9 out of 15 flocks were positive for AI H5N8 virus (1 turkey + 1 duck + 7 broilers). Partial sequencing for selected isolates of six ND, five IB, four H9N2, and three H5N8 viruses was applied, then nucleotide sequences were accessed on GenBank. Six ND isolates belonged to genotype VII viruses circulating in Egypt. Two IB isolates were related to the classical strain circulating in Egypt, while the other three IB isolates belonged to EGY/Variant II. Four H9N2 AI isolates were related to G1-lineage of H9 viruses circulating in the Middle East and Egypt. Three H5N8 AI isolates belonged to the highly diverse clade 2.3.4.4.b viruses circulating in Egypt. It was concluded that ND and IB viruses isolated in this study were not related to their vaccinal strains. Also, AI H5N8 circulating alone in affected flocks while AI H9N2 circulating alone and/or mixed with either IB or ND viruses. Finally, there is a need to devise a complete strategy to control the isolated respiratory viruses on the north coast of Egypt.

**Keywords:** Poultry, Respiratory, RRT-PCR, Sequence, Viruses.

[Full text-PDF] [XML] [Crossref Metadata]



Isolation and Identification of Newcastle Disease Virus From Ducks Sold at Traditional Livestock Market Center in Indonesia

Azizah AN, Anwar Ch, and Rahardjo AP (2021). Isolation and Identification of Newcastle Disease Virus from Ducks Sold at Traditional Livestock Market Center in Indonesia. *J. World Poultr. Res.*, 11 (1): 96-100. DOI: <https://dx.doi.org/10.36380/jwpr.2021.12>

## Research Paper

### Isolation and Identification of Newcastle Disease Virus from Ducks Sold at Traditional Livestock Market Center in Indonesia.

Azizah AN, Anwar Ch, and Rahardjo AP.

*J. World Poultr. Res.* 11(1): 96-100, 2021; pii: S2322455X2100012-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.12>

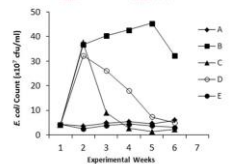
**ABSTRACT:** Newcastle disease (ND) is one of the important infectious diseases in the poultry industry. The traditional poultry markets have great potential in ND transmission. The ducks infected by the ND virus rarely show clinical symptoms, thus they can potentially spread the disease to other fowls. The current study aimed to isolate and identify the ND virus from ducks in a traditional live bird market center in East Java, Indonesia. Cloacal swab samples were taken from 300 ducks. The study consisted of 100 pooled samples, each containing a cloacal swab sample obtained from 3 individual ducks. The samples were inoculated in specific antibody-negative embryonated chicken eggs for 8-10 days. Hemagglutination and hemagglutination inhibition tests were performed for confirmation and identification of ND virus. Based on the result of the current study, out of 100 pooled samples, there were three to nine ducks infected with the ND virus.

**Keywords:** Cloaca Swab, Duck, Livestock, Newcastle Disease

[Full text-PDF] [XML] [Crossref Metadata]



A = healthy control, B= infected and untreated chicks, C= infected and treated with norfloxacin, D= infected and treated with *Lactobacillus casei*, E= *Lactobacillus casei* prophylaxis.



Ikele OM, Ezeonu IM, and Umeh ChN (2021). Control of Intestinal *E. coli* Infection in Broiler Chicks Using *Lactobacillus casei* Isolated from *Nono*. *J. World Poultr. Res.*, 11 (1): 101-109. DOI: <https://dx.doi.org/10.36380/jwpr.2021.13>

## Research Paper

### Control of Intestinal *E. coli* Infection in Broiler Chicks Using *Lactobacillus casei* Isolated from *Nono*.

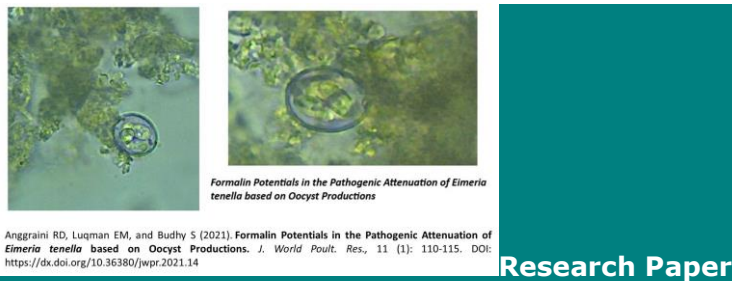
Ikele OM, Ezeonu IM, and Umeh ChN.

*J. World Poultr. Res.* 11(1): 101-109, 2021; pii: S2322455X2100013-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.13>

**ABSTRACT:** The current study aimed to evaluate whether the probiotic *Lactobacillus casei* could be effective in controlling chicken intestinal colibacillosis. Avian pathogenic *Escherichia coli* (*E. coli*.) and *Lactobacillus casei* (*L. casei*) isolates were obtained from *nono* (a sour milk product produced by Fulani tribe of Nigeria), and were used for the chicken infection and probiotic treatment, respectively. The experimental design was conducted on three-week-old broiler chicks, which were divided into five groups, namely A (healthy control), B (infected without treatment), C (infected and treated with antibiotic), D (infected and treated with *L. casei*), and E (initially given *L. casei* before infecting with *E. coli*). Groups C and D were treated using 15 g/L norfloxacin and 1.5 ml of  $1.1 \times 10^9$  cfu/ml *L. casei*, respectively. Group E was given the oral infusion of 1.5 ml of  $1.1 \times 10^9$  cfu/ml *L. casei* before infection with 1.5 ml of  $1.3 \times 10^7$  cfu/ml avian pathogenic *E. coli*. Weight, hematological parameters, liver function, and fecal *E. coli* counts of the chicks were monitored and used to evaluate the level of protection elicited by the probiotic organism. There was weight gain in chicken groups, except for group B. There was a significant difference in the sodium, chlorine, and bicarbonate levels amongst the groups. The hematological profile revealed a significant difference in the hemoglobin, white blood cells, lymphocyte, and neutrophil counts of the chicken groups. Assessment of liver enzymes showed no significant difference amongst the chick groups except in group B. Similar results were obtained for the urea, creatinine, and C-reactive protein levels. The microbial tests revealed a decrease in the total *E. coli* count for groups C, D, and E. The results of the current study indicated that *L. casei* could be used as a probiotic

[Full text-PDF] [XML] [Crossref Metadata]



## Formalin Potentials in the Pathogenic Attenuation of *Eimeria tenella* based on Oocyst Productions.

Anggraini RD, Luqman EM, and Budhy S.

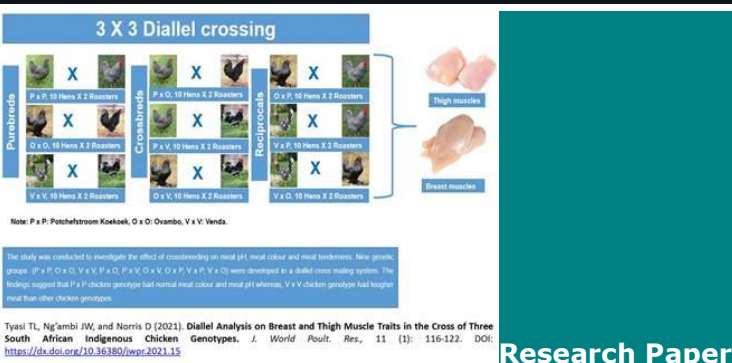
*J. World Poult. Res.* 11(1): 110-115, 2021; pii: S2322455X2100014-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.14>

**ABSTRACT:** Coccidiosis is a disease found in poultry caused by parasitic protozoa, namely *Eimeria tenella* (*E. tenella*), which may lead to high rates of morbidity and mortality. To prevent coccidiosis, vaccination is required to inactivate and attenuate *E. tenella* protozoa. One of the compounds applied for attenuation is formaldehyde. Formaldehyde reduces the pathogenicity of an organism by creating rigidity in its structure. As a result, the organism cannot inflict disease and has a higher impact on building antibodies although it is still alive. The current research was an experimental study aimed to determine the formalin potential in attenuation of *E. tenella* pathogenesis in terms of oocyst production. The present study was conducted using the completely randomized design method. A total number of 25 broiler chickens were applied and their feces were tested to observe oocysts production and clinical symptoms. The obtained data would be analyzed by the ANOVA statistical test. The treatment groups presented clinical symptoms of *E. tenella* infection. The number of oocysts in treatment group I fluctuated from the lowest number which was zero on day five and then increased by day six, seven, and eight and it has reached the peak with the most significant amount of 4,050,460 oocysts on day nine. The treatment group II with the same condition reached its peak with the highest number of 1,363,160 oocysts on day nine. The treatment group III peaked with the most significant number of 618,960 oocysts on day nine. In addition, the treatment IV group attained the apex with the highest number of 719,480 oocysts on day nine. Meanwhile, the treatment V group reached the highest number of 284,200 oocysts on day nine. The difference in formalin concentration affected the amount of *E. tenella* oocyst production of broiler chickens. Formalin soaking with a concentration of 1.2% was the most optimal concentration to attenuate *E. tenella*.

**Keywords:** Broiler chicken, *Eimeria tenella*, Formalin, Oocyst.

[Full text-PDF] [XML] [Crossref Metadata]



## Diallel Analysis on Breast and Thigh Muscle Traits in the Cross of Three South African Indigenous Chicken Genotypes.

Tyasi TL, Ng'ambi JW, and Norris D.

*J. World Poult. Res.* 11(1): 116-122, 2021; pii: S2322455X2100015-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.15>



**ABSTRACT:** The present study aimed to estimate carcass characteristics of pure and crossbred chickens produced from three parental populations. A 3 × 3 complete diallel mating system involving three indigenous breeds, namely Potchefstroom Koekoek (P), Venda (V), and Ovambo (O), was used to produce three purebred (P × P, V × V, O × O), three crossbreds (P × O, P × V, O × V) and three reciprocals (O × P, V × P, V × O). The nine genetic groups were reared from hatch to 10 weeks of age in an open house with deep litter. At 10 weeks of age, six chickens per genetic group were randomly selected for slaughter. After slaughtering the breast and thigh muscles samples for analysis of the carcass characteristics (Meat colour, meat pH, and Shear force). The results showed that the Potchefstroom Koekoek breed had higher values in all colour indicators, L\* (lightness), a\* (redness), and b\* (yellowness), compared to the other chicken breeds. The Potchefstroom Koekoek and P × O breed had higher pH values ranging from 5.66 to 6 at two hours post-slaughter and from 5.54 to 6.38 at 24 hours post-slaughter. The pH declines in all the nine genetic groups after two to 24 hours, with the exception of the crossbred P × O, which increased from 6.06 to 6.38. In terms of shear force, the O × P had the highest shear value, ranging from 35.89N to 74.80N, compared to other genetic groups. Potchefstroom Koekoek had normal meat colour and pH, whereas the Venda breed had tougher meat than other genotypes. The results of the present study might be useful for local chicken farmers to improve carcass traits.

**Keywords:** Crossbred, Meat colour, Meat pH, Purebred, Shear force.

[Full text-PDF] [XML] [Crossref Metadata]

**Research Paper**

### Histopathology Description of Chicken Liver Infected by L2 *Toxocara Vitulorum*.

Auliyah R, Kusnoto, and Hamid IS.

*J. World Poult. Res.* 11(1): 123-128, 2021; pii: S2322455X2100016-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.16>

**ABSTRACT:** Transmission of *Toxocara vitulorum* Infection causes a decrease in livestock productivity and results in various types of diseases in humans. Chickens are one of the parasitic hosts of toxocarasis which has the potential for transmission of toxocarasis to humans. The main location affected by *T. vitulorum* larval infection is the liver. The current study aimed to analyze the description of histopathological changes in the liver of broiler chickens infected by L2 *Toxocara vitulorum*. The present study was a true experiment using a completely randomized design. A total number of 28 broiler chickens aged 14 days were selected as the sample in this study. Samples were infected using L2 *Toxocara vitulorum* larvae and were grouped in accordance with observations of the 1, 2, 3, 7, 14, and 21 days after the larvae were given to the samples. *Toxocara vitulorum* larval infection caused changes in histopathological features of broilers chickens. This infection caused hydropic inflammation and degeneration of liver cells, cholangitis, and eventually necrosis of the cells. Exposure to infection over a long period of time can worsen liver cell and other organ damages as well as increasing the potential for the transmission of *Toxocara vitulorum* larvae.

**Keywords:** Chicken, Histopathology of liver, Infection, *Toxocara vitulorum*.

[Full text-PDF] [XML] [Crossref Metadata]

**Research Paper**

### Effects of Red and Blue Light during the Incubation of Turkey Eggs on Hatchability Performance and Expression Pattern of Some Myogenic Regulatory Genes.

Abd El Naby WSH, Basha HA, Ibrahim SE, and Abo-Samaha MI.

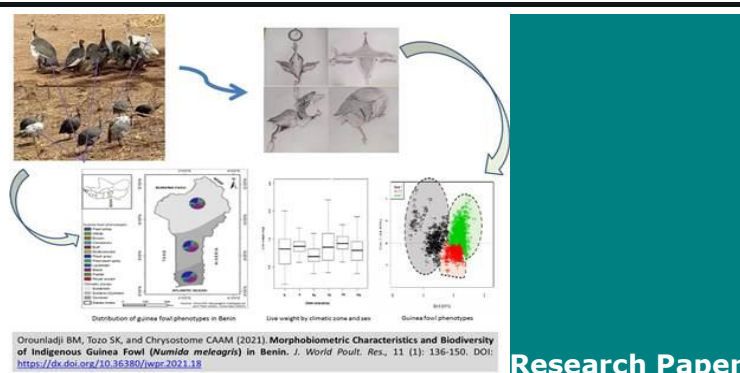
*J. World Poult. Res.* 11(1): 129-135, 2021; pii: S2322455X2100017-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.17>

**ABSTRACT:** The present study aimed to investigate the effects of different light colors on hatching potential traits, including egg weight loss, scientific and commercial hatchability, mortality percentages, hatching weight as well as mRNA expression levels of some muscle growth marker genes (Myogenin, MyoD1, and FGF2) of pectoralis muscle in hatched and non-hatched non-pipped Black Bronze turkey chicks. A total of 1500 hatching Black Bronze turkey eggs were assigned equally to three incubation treatment groups, namely dark (control group), red, and blue LED light (treated groups) for 25 days of the incubation period. Results indicated that colored lighting stimuli (red and blue) significantly affected hatching capability. This issue could also affect the expression of muscle growth marker genes in hatched and non-hatched non-pipped turkey chicks. Incubation of turkey eggs under red or blue LED light showed an insignificant effect on mortality percentages. It can be concluded that the use of a red or blue light system during turkey eggs' incubation could improve hatchability via upregulating the expression of muscle growth marker genes.

**Keywords:** Hatchability, Incubation, Light color, Marker Gene expression, Turkey

[Full text-PDF] [XML] [Crossref Metadata]



## Morphobiometric Characteristics and Biodiversity of Indigenous Guinea Fowl (*Numida meleagris*) in Benin.

Orounladji BM, Tozo SK, and Chrysostome CAAM.

*J. World Poult. Res.* 11(1): 136-150, 2021; pii: S2322455X2100018-11

DOI: <https://dx.doi.org/10.36380/jwpr.2021.18>

**ABSTRACT:** The present study aimed to describe the morphobiometric characteristics of indigenous guinea fowl (*Numida meleagris*) populations in Benin. The current study was carried out on 1320 (529 males and 791 females) adult (at least 24 weeks old) indigenous guinea fowls from three climatic zones (Sudanian, Sudano-Guinean, and Guinean) of Benin. Each guinea fowl was subjected to a direct phenotypic description, biometric measurements, and photography. The results showed that the plumage coloration of indigenous guinea fowl in Benin was significantly diverse, but the most widespread plumage colors were pearl grey (30%), black (29.5%), and cinnamon (9.8%). The most common beak colors were grey (64.9%) and yellow-orange (24.8%). The eyes were predominantly black-white (67.1%). Grey-orange (33.7%), grey (32%), and black-orange (21%) colorations were more represented on the shanks with wattles relatively dominated by red-white (59.4%) and white-red (30.5%). The average live weight of guinea fowl was 1.34 kg in males which was 4.38% heavier than females. All the biometric measurements were significantly higher in males. The live weights of guinea fowl in the Sudanian zone ( $1.40 \pm 0.18$  kg) were higher than those of guinea fowl found in the Sudano-Guinean zone ( $1.27 \pm 0.24$  kg) and Guinean zone ( $1.33 \pm 0.28$  kg). Principal Component Analysis indicated that three distinct groups of guinea fowl can be formed based on their biometric measurements (live weight, chest circumference, body length, drumstick length, shank length, shank diameter, and wingspan). The phenotypes' diversity was relatively abundant (1-Hill: 0.69) in all climatic zones. The phenotypic biodiversity observed in the populations of indigenous guinea fowl in Benin can guide farmers to select specific phenotypes to meet consumer preferences.

**Keywords:** Benin, Biodiversity, Climatic zone, Indigenous guinea fowl, Phenotypic characteristic.

[Full text-PDF] [XML] [Crossref Metadata]



# Histopathology Description of Chicken Liver Infected by L2 *Toxocara Vitulorum*

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<sup>1</sup> Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia

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Received: 21 Jan. 2021

Accepted: 10 Mar. 2021

## ABSTRACT

Transmission of *Toxocara vitulorum* Infection causes a decrease in livestock productivity and results in various types of diseases in humans. Chickens are one of the parasitic hosts of toxocariasis which has the potential for transmission of toxocariasis to humans. The main location affected by *T. vitulorum* larval infection is the liver. The current study aimed to analyze the description of histopathological changes in the liver of broiler chickens infected by L2 *Toxocara vitulorum*. The present study was a true experiment using a completely randomized design. A total number of 28 broiler chickens aged 14 days were selected as the sample in this study. Samples were infected using L2 *Toxocara vitulorum* larvae and were grouped in accordance with observations of the 1, 2, 3, 7, 14, and 21 days after the larvae were given to the samples. *Toxocara vitulorum* larval infection caused changes in histopathological features of broilers chickens. This infection caused hydropic inflammation and degeneration of liver cells, cholangitis, and eventually necrosis of the cells. Exposure to infection over a long period of time can worsen liver cell and other organ damages as well as increasing the potential for the transmission of *Toxocara vitulorum* larvae.

**Keywords:** Chicken, Histopathology of liver, Infection, *Toxocara vitulorum*

## INTRODUCTION

*Toxocariasis* is one of the worm-originating diseases that can attack ruminants, especially calves of cows and buffaloes and the mothers (Hübner et al., 2001). *Toxocara vitulorum* which attacks cows at all ages can be transmitted through food boxes or placenta that can infect the fetus in the womb (Levine, 1995). *Toxocara vitulorum* is commonly found in tropical and subtropical climates (Starke et al., 1996). This infection leads to a reduction in livestock productivity, which will be a financial burden for farmers if not controlled. In addition, *T. vitulorum* infection causes anorexia, stomach pain, diarrhea, constipation, dehydration, bad breath, and also a decrease in the body weight of cattle (Raza et al., 2013).

Humans or animals that consume raw or undercooked liver of paratenic hosts of *Toxocara* spp. are the potential to being contaminated with toxocariasis (Yoshikawa et al., 2008). Some paratenic hosts of toxocariasis are mice, rats, pigs, birds, chickens, humans, and other mammals (Azizi et al., 2007; Yoshikawa et al., 2008; Raza et al., 2013). Larvae can move to various

tissues and survive for a long period of time (Azizi et al., 2007; Strube et al., 2013). The movement of larvae into the tissues (lung, liver, and kidney) or milk is thought to be a medium of transmission to humans (Kusnoto et al., 2005). The consumption amount of raw or undercooked meat increases the prevalence of toxocariasis cases (Taira et al., 2011) leading to human zoonosis diseases, such as visceral larva migrans (VLM) and ocular larva migrans (OLM).

*T. vitulorum* larvae can cause liver and lung lesions, inflammation of lymph nodes, as well as eosinophilia during the life cycle of the parasite (Abbott et al., 2006; Khan et al., 2007). *Toxocara* spp. larvae migrate to the liver through the porta hepatica systems and cause hepatomegaly which is a common phenomenon (Soulsby and Monnig, 1982). In humans, The human infection of *Toxocara* spp. leads to hepatocellular necrosis and inflammatory reactions (Hübner et al., 2001). On the other hand, histopathological examination of visceral organs using helminthiasis has not been performed much, especially to see the histopathological picture of the liver as the site of second-stage *T. vitulorum* larvae migration in

chickens as paratenic host, where parasites can live but cannot develop into adulthood (Cardillo *et al.*, 2008). Therefore, this study was conducted to describe the histopathological changes in the liver of broiler chickens after being infected by L2 *Toxocara vitulorum*.

## MATERIALS AND METHODS

The present study was a true experiment using a completely randomized design performed at the Helminthology Laboratory of the Parasitology Department of Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, in 2020. The liver histopathology examination was carried out in the Pathology Laboratory. Three variables have been investigated in the current study. The histopathological image of the liver (degeneration and necrosis of hepatocytes) was considered as the dependent variable, the effective dose of L<sub>2</sub> *T. vitulorum* and liver sampling time were taken into account as independent variables. Finally, controlled variables included strain, sex, age, feed, and also environmental conditions of chickens.

### Experimental design

Broiler infection involves eggs containing L2 *T. vitulorum*. Phosphate Buffer Saline (PBS) (Sigma Co.) was used as the media of L2 *T. vitulorum*, while the protozoa growth and other microorganisms were avoided using formalin 0.5-1%. In addition, Trypsin 1% was used for releasing L2 *T. vitulorum* from the formed liver tissue.

### Isolation and preparation of *Toxocara vitulorum* eggs

Infective eggs of *T. vitulorum* worm inoculant material comes from the intestine of 10 cows contaminated by *toxocariasis*, during visiting the slaughter-house. Worms were washed in 0.85 % saline to remove debris, and they were fixed into 70 % ethanol. The worms were then cleaned with aquadest and transferred to a container containing PBS solution as a development medium. After that, the worms were incubated at 37°C for up to three days in order to lay *T. vitulorum* eggs. The *T. vitulorum* egg retrieval was performed through the worm's reproductive tract by the surgical process. Subsequently, a gradient preparation technique was used to separate the dirty debris from the eggs.

### *Toxocara vitulorum* eggs fertilization

Egg fertilization was carried out in PBS medium with the addition of five drops of formaldehyde 10%. This addition served to prevent the interference of other microbes with the growth of *T. vitulorum* eggs until the

first (L1) and second larvae were obtained (L2). The development of worm eggs was observed using a dissecting microscope (Olympus upright microscope) and documented in the form of photographs on a regular basis. The process took 21-28 days at room temperature until the egg developed into L2 (Kusnoto *et al.*, 2011).

### Calculation of *Toxocara vitulorum* eggs

The egg calculation was carried out using a modified calculation of the worm eggs per gram excretion introduced in the Lucient Brumpt method (Kusnoto *et al.*, 2007). An amount of 1 ml of *T. vitulorum* egg suspension from the culture media was then taken and diluted 10 times until 10 ml of suspension achieved. Then, 1 ml of suspension was taken by means of a Pasteur pipette to calculate the number of drops for every 1 ml of suspension. One drop of the suspension was put on a glass object and then examined through a light microscope with 100× magnification. Eggs that appeared through the microscopic magnification were counted using the formula which is written below (Kusnoto *et al.*, 2007).

Number of eggs: Number of drops per ml (N) × number of worm eggs per drop (n) × number of dilutions

### Treatment of experimental animals

A total number of 28 broiler chickens with body weights of 100-200 gr were selected as experimental animals of the current study. The chickens were raised on the farm and the floor. The adaptation period was one week. There was no available information about the vaccination program since the study was performed during rearing. Broilers were required to be 14 days old for deviation to seven treatment groups. The chickens were quarantined (A week) before being randomly divided into seven treatments with four replications in each. The chickens were feeding *ad-libitum* every afternoon and morning with strict hygiene. Then, each broiler chicken was infected by using 3000 eggs containing *T. vitulorum* second stage larvae (L2) when it was added to the food. The broiler chicken groups were divided into six groups (Azizi *et al.*, 2007; Taira *et al.*, 2011). The broiler chickens in the control group (K) were not infected with *T. vitulorum*. The P1 consisted of broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized a day after infection. The broilers in P2 group were infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized two days after infection. The P3 group entailed broilers infected with L<sub>2</sub> *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized three days after infection. The P4 had broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized seven days after infection. Moreover, P5 was



composed of broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized 14 days after infection. Finally, the P6 group encompassed broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized 21 days after infection.

### Liver extraction

Liver extraction for histopathological preparation was carried out 1, 2, 3, 7, 14, and 21 days after L2 *T. vitulorum* infection. Extraction of the chicken liver was done after euthanasia and surgery. Broiler's liver organs were stored in aquadest and formalin 10%. Chicken's livers were cleaned with physiological NaCl then put in a plastic pot containing aquadest and formalin 10%, and subsequently stored for 24 hours before making the histopathological preparations.

### Examination of preparations

The materials used for liver histopathological preparation were multilevel ethanol (70%, 80%, 90%, and absolute), formalin 10% added to the solution, ether, physiological saline (NaCl 0.9%), paraffin, entellan (transparent adhesive), Harris's Haematoxylin-Eosin double coloring, emersion oil, and xylol. Examination of preparations was performed using a light microscope with 400× magnification of five different fields of view (LP) for each sample. The observed changes included degeneration, the swelling of cell size due to vacuoles in the cytoplasm, Infiltration of inflammatory cells around the central vein, whether porta hepatis or sinusoid. Subsequent examination of preparations was assessed according to the Knodell score method (Knodell et al., 2019).

### Statistical analysis

The research data including the histopathological score of liver cells of chickens were analyzed using Kruskal Wallis test, then continued with the Z-test. Differences were considered significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

The present study obtained the results from the observation of isolated *T. vitulorum* worm eggs from adult worms that were fertilized and incubated for about one month. This process also obtained a second-stage larvae (L2) (Figure1). The results of the treatment on broilers microscopically demonstrated a histopathological change in the chicken liver after being infected with L2 *T. vitulorum*. Non-parametric Kruskal Wallis test indicated a significant difference ( $p < 0.05$ ) for each treatment in broilers (Table 1). Provision of infective larval infections

(L<sub>2</sub>) *T. vitulorum* affects the histopathology of broiler chickens' liver. This study found a significant difference between the control group (K) and treatment groups which were euthanized 1- 21 days post-L2 infection ( $p < 0.05$ ). The obtained scores were then followed by a multiple comparison test (Z test) to determine the order of the change rate in the liver histopathological pictures among the seven treatment groups.

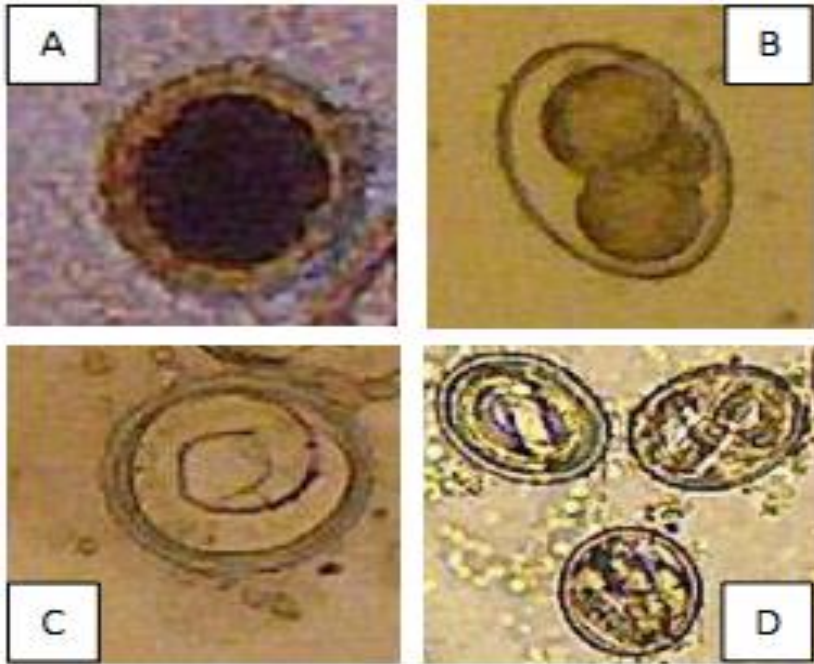
Histopathological pictures of the liver tissue in the treatment groups presented damage due to hydropic degeneration (cloudy swelling), necrosis, inflammation, and cholangitis. The Z test indicated significant differences in the treatment groups P1, P3, P4, P5, and P6 with the control group (K). However, there was a change in histopathological features in P2 which were not significantly different from the control group. Group P6 represented the worst results, compared to other treatment groups (Figure 2).

In Figure 3, part A, hepatocytes were normal (blue sign) and did not appear to have inflammation and degeneration, and ductal images were still normal (green signs). In figure B, the cholangitis in the P6 group was characterized by inflammatory cells (yellow marking) and epithelial proliferation (red marks) of the bile duct. In figure C, the black mark referred to the presence of hydropic degeneration, and cytoplasm appeared turbid (cloudy swelling) and the green mark referred to the necrosis of the nucleus which appeared to be picnotic. The yellow mark in figure D indicated inflammation around the portal area.

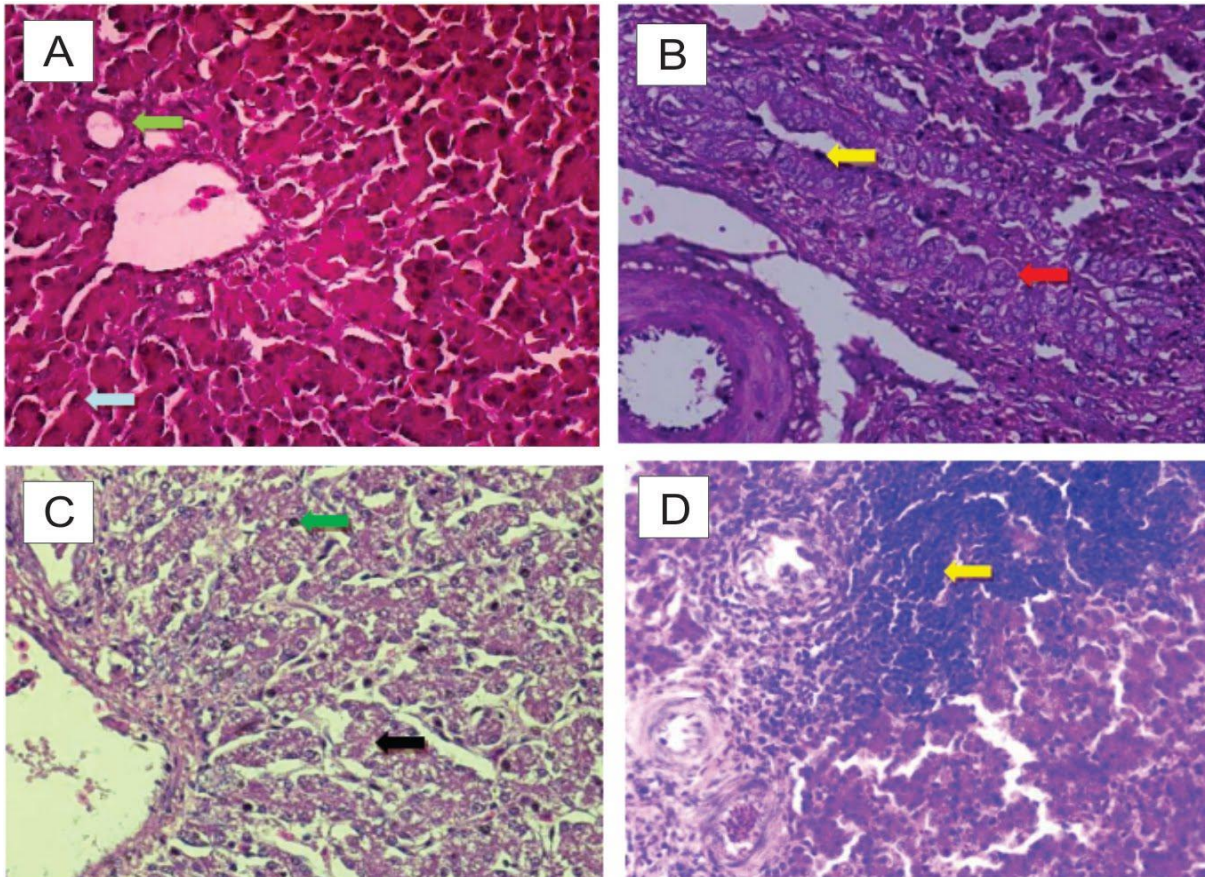
**Table 1.** Statistical results on the extent of liver damage to the broiler chickens infected by *T. vitulorum*

Treatment	Liver Damage Value (Mean Rank ± SE <sup>1</sup> )
K	2.50 <sup>d</sup> ± 0.289
P1	15.50 <sup>b</sup> ± 1.472
P2	9.63 <sup>c</sup> ± 0.816
P3	12.50 <sup>bc</sup> ± 1.443
P4	14.75 <sup>b</sup> ± 1.323
P5	21.25 <sup>ab</sup> ± 1.190
P6	25.38 <sup>a</sup> ± 0.645

a-d: different superscripts in the same column show significant differences ( $p < 0.05$ ) <sup>1</sup>SE: Standard Error. K: control group, broilers were not infected with *T. vitulorum*. P1: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized a day after infection. P2: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized two days after infection. P3: Broilers were infected with L<sub>2</sub> *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized three days after infection. P4: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized seven days after infection. P5: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized 14 days after infection. P6: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized 21 days after infection.



**Figure 1.** The results of identification of the *Toxocara vitulorum* worm eggs and their development up to L2 stage with 100× Magnification, A: worm eggs (1 cell), B: morula, C: L1, D: L2



**Figure 3.** Histopathological picture of changes in different groups of infected broiler chickens' liver with L2 *Toxocara vitulorum*. Haematoxylin-Eosin coloring, 400× Zoom. A: control group; B: Chicken liver cholangitis P6, C: liver degeneration and necrosis P6, D: chicken liver inflammation P6



## DISCUSSION

The oral administration of L2 *T. vitulorum* caused a significant change in the liver histopathology picture of broiler chickens ( $p < 0.05$ ). This was due to the migration of larvae into the tissue. However, the larvae were not always found on liver histopathological examination (Fenoy et al., 2001). The orally administrated 3,000 eggs presented a white spot on the hepatic surface of the chickens indicating the presence of necrotic foci, eosinophil infiltration, and some lymphocytes around the necrotic area (Azizi et al., 2007; Taira et al., 2011).

Infective eggs of *T. vitulorum* hatched within 2 hours followed by penetration into the intestinal wall to reach the liver through the porta hepatica system. The life cycle of *Toxocara* spp. involves a phase of migration in tissue at every stage, starting from the egg, larvae, and adult stages. Every stage of *Toxocara* spp. growth has different antigenic devices and immunogenicity in triggering the formation of antibodies. Infective larval migration can cause histopathological changes in the cells of organs (Santos et al., 2017).

The liver experienced severe damage in the first post-infection day with the occurrence of degeneration, necrosis, severe inflammation, and cholangitis. On the second day, the liver was damaged but there were no significant differences in histopathological features which were found between treatment groups and the control group. L2 *Toxocara* spp. was most commonly found in the liver on the first day after infection and L2 migrated to another site on the second day (Taira et al., 2011). On the second post-infection day, the L2 *Toxocara* spp. was mostly found on the pulmonary of the chickens. Injuries of the liver cells were reversible and the cell would return to its original stable state within a certain time limit (Kumar et al., 2013). Histopathological picture of chicken liver in P3, P4, P5, and P6 groups indicated liver damage, especially around the central port and venous regions. *Toxocara* spp. migrated to other tissues through the circulatory system. The route of migration through the bloodstream can subsequently cause hemorrhage and multifocal necrosis in the liver. Inflammatory cell findings and epithelial proliferation in the bile duct were also observed in all treatment groups. Infective larvae *T. vitulorum* can migrate through the portal vein and then enter the bile duct through enterohepatic circulation (Azizi et al., 2007).

Histopathological pattern of liver cells infected with L2 *T. vitulorum* experienced degeneration, swelling and

was accompanied by necrosis, inflammation, and cholangitis. *Toxocara* spp. larvae secrete metabolic material that caused injury to liver cells. The products or secretions of infectious organisms are toxic to the metabolism or integrity of the cell membrane (Underwood, 1996). Degenerated liver cells experiencing cloudy swelling, microscopically present the granular cytoplasm and appeared to be foggy (Thomson, 1984). This change reveals that when water accumulates in the cytoplasm, cytoplasmic organelles also absorb water which causes swelling of the mitochondria and enlargement of the rough endoplasmic reticulum accompanied by the loss of ribosomes (Cotran et al., 1994).

Liver cell necrosis is characterized by three changes in the cell nucleus, including picnosis which means the cell nucleus appears round, dark, and smaller than the normal cell nucleus, karyorrhexis is splitting the cell nucleus into several parts, and karyolysis means when the cellular nucleus chromatin disappears and leaves holes in the cell (Thomson, 1984). L2 *T. vitulorum* infection in experimental animals caused cell necrosis and disabled the cells to stimulate changes so that eventually cell death occurred. This death is a result of releasing several enzymes, such as ATP-ase, phospholipase, protease, and endonuclease. Great or lethal lesions lead to irreversible cell damages because the cell cannot defend itself against injury.

*Toxocara* spp. larvae secrete metabolic material that increases the production of eosinophils as an immune reaction. Cellular activity and pressure of infection can stimulate microbicidal secretions, effectors, and inflammatory mediators. This pressure responds to cells to protect and fight unwanted conditions by minimizing damage and maintaining the integrity of the host tissue. Endoplasmic reticulum and mitochondrial tissue are key cellular organelles which give signals to cellular pressure (Abbas and Lichtman, 2003). Cholangitis is inflammation of the bile wall due to lumen infection. This situation can originate from any lesion that blocks the bile duct.

Therefore, L2 *L. vitulorum* can migrate to various organs and cause damage, hence some prevention can be done by health workers such as conducting training and counseling on the importance of cleanliness and environmental management. In addition, it is also necessary to provide support and regular assistance to farmers. This aims to minimize the spread of infection by reporting the cases to health workers.

## CONCLUSION

Infective larvae of (L2) *Toxocara vitulorum* administered orally could provide a change in the histopathological picture of broiler chicken's liver. Liver cell damages included cell degeneration, inflammatory cell infiltration, necrosis, and cholangitis. The P6 treatment group presented the most damage, compared to the other treatment groups, since the liver cells and other organs in chickens were exposed to toxic metabolic material released from the *Toxocara vitulorum* larvae during a longer period of time.

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