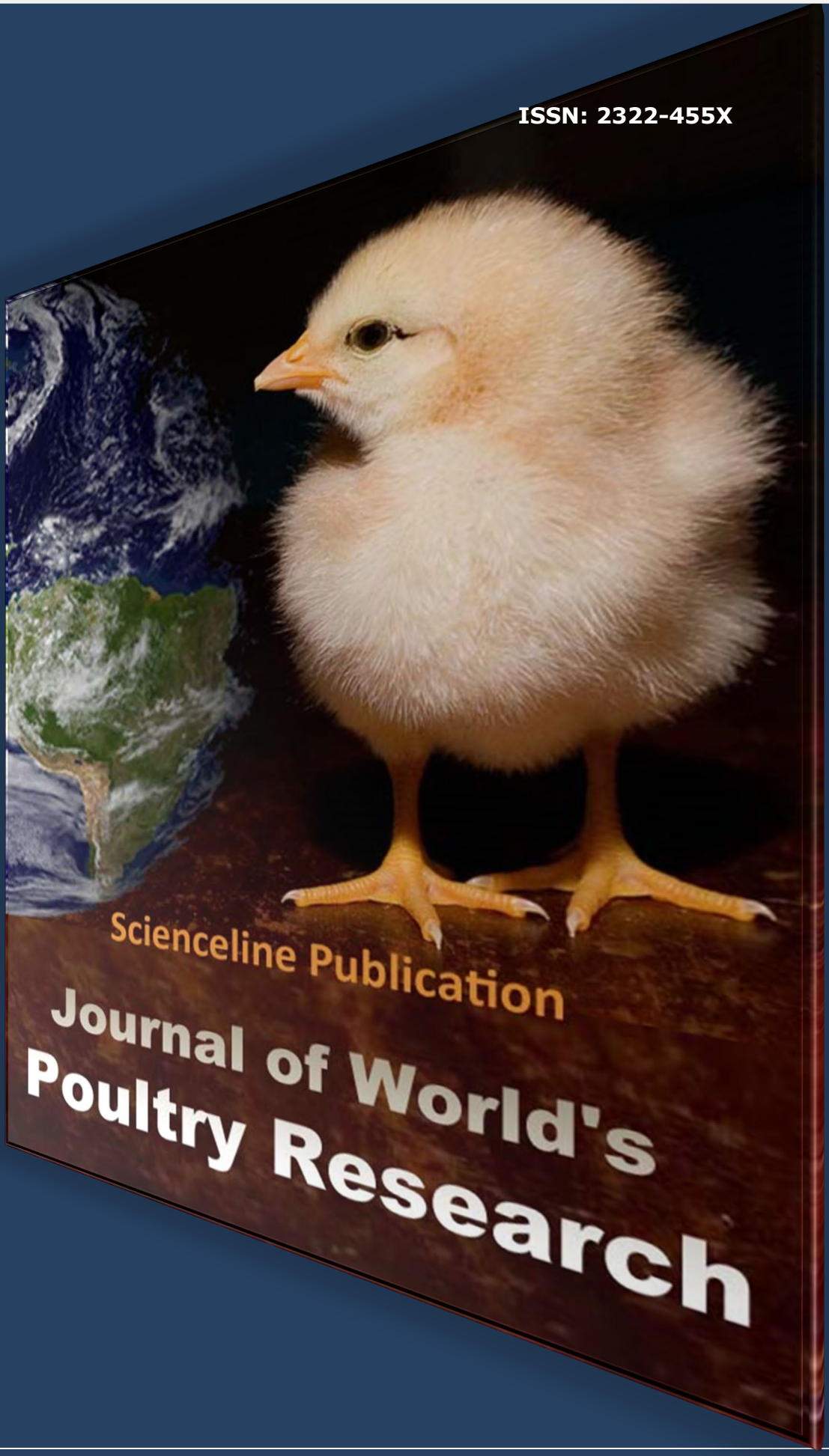
Journal of World's Poultry Research

BOOKLET

ISSN: 2322-455X



Volume 12, Issue 2, June 2022

JWPR

Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12 (2): 77-132, June 25, 2022

Editors-in-Chief

Managing Editor

Associate Editors

Language Editor

Statistical Editor

Technical Editor

Editorial Team

Advisory Board

TABLE OF CONTENT

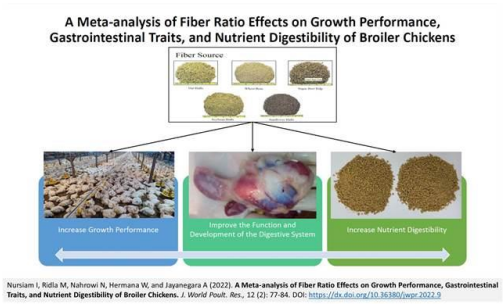
Volume 12 (2); June 25, 2022

Research Paper

A Meta-analysis of Fiber Ratio Effects on Growth Performance, Gastrointestinal Traits, and Nutrient Digestibility of Broiler Chickens

Nursiam I, Ridla M, Nahrowi N, Hermana W, and Jayanegara A.

J. World Poult. Res. 12(2): 77-84, 2022; pii: S2322455X2200009-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.9



ABSTRACT: Fiber is one of the essential nutrients for broiler chickens. This meta-analysis was carried out to investigate the impacts of fiber fraction ratio on broiler chickens growth performance, digestive characteristics, and nutritional digestibility. The database was compiled from 15 publications reports on the addition of fiber sources in broilers feed. To analyze the effect of acid detergent fiber (ADF) / neutral detergent fiber (NDF) ratio, the mixed model technique was utilized, with ADF/NDF ratio in the feed as a fixed effect and the experiment as a random effect. The ADF/NDF ratio in the feed had no effect on average daily gain, average daily feed intake, and feed per gain ratio in this research. Moreover, a decrease in ADF/NDF ratio in broiler chicken feed increased the relative weight of the gizzard. The relative weight and length of the small intestine and cecum were not affected by the ADF/NDF ratio in the feed. The ADF/NDF ratio in feed enhanced ileal digestibility and total tract apparent retention of most nutrients. The ADF/NDF ratio in the feed had no effect on the jejunal morphology. The minimum ADF/NDF ratio of 0.37 in the feed led to the maximum growth performance, digestive tract development, and optimal nutrient digestibility. In conclusion, controlling the ratio of fiber fraction in broiler chickens feed can improve broiler performance in the non-antibiotic growth promoters era.
Keywords: Broilers chickens, Fiber fraction, Meta-analysis, Performances
[Full text-PDF]

Research Paper

Effect of Partial Replacement of Yellow Corn by Mango Seed Kernel on Productive Performance, Egg Quality, and Blood Constituents of Laying Hens

Eid Farag ME, Elsayed Moustafa K-E.M, El-Saadany ASh, and Abu Hafsa SH.

J. World Poult. Res. 12(2): 85-97, 2022; pii: S2322455X2200010-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.10

ABSTRACT: Corn is the main energy source in most poultry feed. Due to rapid climate change, corn production cannot keep up with the demand for food and industrial applications. This necessitated the search for alternatives, such as agro-industrial by-products like mango seed kernel, which is a good source of carbohydrates and protein and can wholly or partly replace corn. The purpose of this study was to investigate the effect of partial replacement of yellow corn (YC) with soaked mango seed kernel (SMSK) on productive and reproductive performance, egg quality, blood biochemistry, hematological parameters, and antioxidants status of local laying hens. A total of 120 local *Gimmizah* breeds (108 females and 12 males) at 32 weeks of age were randomly assigned to four dietary treatments with three replicates (9 hens + 1 male per replicate). The treatments were YC replaced by SMSK at 0, 10, 15, and 20% levels in the hen diets for 12 weeks. Replacing YC with SMSK increased egg production, weight and number of eggs, and egg mass, and improved feed conversion ratio, but feed intake was not affected. Replacement of yellow corn with SMSK did not affect egg quality parameters. The hens in the SMSK 10% and SMSK 15% groups had the highest fertility, hatchability, post-hatch chick weight, and number followed by those in the SMSK 20% group. Groups given varying levels of SMSK had the lowest rate of embryonic mortality. Carcass weight and dressing percentage were positively affected by the 10% and 15% SMSK diet, except for the heart, pancreas, and spleen. Hematological indices were not influenced by dietary SMSK except for higher platelets in the SMSK 20% group. Total protein, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels were similar among treatments. In SMSK groups, serum total cholesterol, triglycerides, and malondialdehyde levels decreased significantly, whereas IgG and catalase levels increased. These findings indicated that SMSK up to 20% could be considered a successful nutritional and health approach and can be partially substituted for YC with no adverse impact on the productive, reproductive and physiological performance of laying hens.

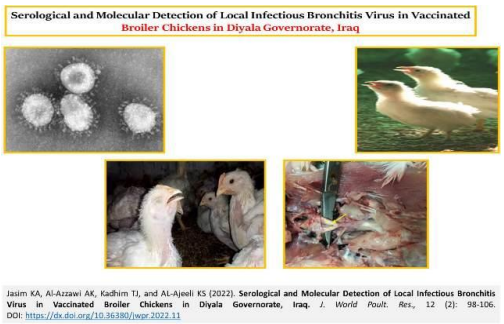
Keywords: Antioxidant status, Egg quality, Egg production, Laying hens, Lipids profile, Mango Seed Kernel, Replacement
[Full text-PDF]

Research Paper

Serological and Molecular Detection of Local Infectious Bronchitis Virus in Vaccinated Broiler Chickens in Diyala Governorate, Iraq

Jasim KA, Al-Azzawi AK, Kadhim TJ, and AL-Ajeeli KS.

J. World Poult. Res. 12(2): 98-106, 2022; pii: S2322455X2200011-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.11



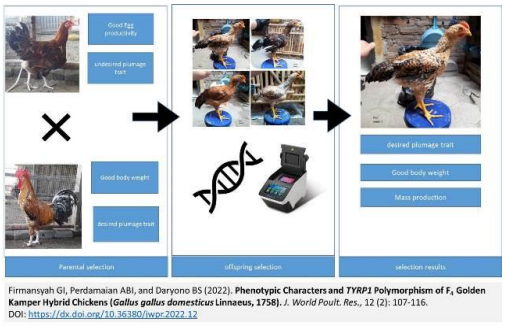
ABSTRACT: The infectious bronchitis virus (IBV) is one of the most important *Coronaviridae* viruses, infecting the upper respiratory tract of chickens and leading to considerable losses in the poultry industry across the globe. Many outbreaks have recently occurred among IBV-vaccinated chicken farms in the Diyala Governorate of Iraq resulting in significant economic losses. As a result, the purpose of the present study was to investigate whether IBV can be a source of infection spread in IBV-vaccinated commercial broiler flock farms in Diyala Governorate. In this regard, ELISA was used as a serological test and RT-PCR as a molecular detection technique. Serum samples were collected from chickens suspected of IBV at 16 and 23 days of age. The results showed a significant increase of IgG antibodies in such serum samples at days 16 and 23 of age indicating the infections of the broilers with IBV. However, at the age of 2-3 weeks, the samples of kidney, liver, trachea, and lungs were collected from clinically and sub-clinically infected flocks, and also postmortem samples were sampled from all farms. Two sets of previously reported primers were created for this purpose in the S1-protein gene region. According to the findings of the present investigation, IBV was found in 83% of samples. Finally, despite immunization with IB4/91, IBV was prevalent in broiler chicken farms in the study area confirmed by serology and molecular biology tests. This finding indicates the possibility of genetic difference between the locally discovered IBV and the administered IBV vaccine. A study on the production of local vaccines can be useful in controlling IBV infections.
Keywords: ELISA, Infectious bronchitis viruses, RT-PCR
[Full text-PDF]

Research Paper

Phenotypic Characters and *TYRP1* Polymorphism of F4 Golden Kamper Hybrid Chickens (*Gallus gallus domesticus* Linnaeus, 1758)

Firmansyah GI, Perdamaian ABI, and Daryono BS.

J. World Poult. Res. 12(2): 107-116, 2021; pii: S2322455X2200012-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.12



ABSTRACT: Golden Kamper is a local meat-typed chicken with four generations of Pelung male and Layer female selective breeding. This chicken has various plumage colors and patterns. Therefore, the desired plumage color is red barred plumage (*B1*). In chickens, the missense mutation in the Tyrosinase-related-proteins 1 (*TYRP1*) causes a chocolate color plumage (*choc*) with an epistatic effect on barred plumage. The current study aimed to observe the growth of 16 chickens from hatching until 49 days of age to investigate the phenotypic characteristics, especially plumage color at 49-day-old chickens, then to determine the effect of the *TYRP1* polymorphism on F_4 Golden Kamper phenotypes. The methods used in this study included selective breeding among F_3 Golden Kamper, collection of F_3 Golden Kamper's eggs, then rearing the day-old chickens of F_4 Golden Kamper. Phenotypic data were collected and blood collection was performed for DNA isolation, DNA amplification, and sequencing. Of 16 F_4 Golden Kamper, all chickens had a uniform comb type of single (rprp, 100%). The produced shank colors were white (31.25%), yellow (62.5%), and blackish gray (6.25%). The plumage colors were red barred (12.5%), white barred (12.5%), brown (68.75%), and chocolate (6.25%). The bodyweight of F_4 Golden Kamper at the age of 7 weeks reached 597.3 g. The morphometric results indicated that F_4 Golden Kamper had the same posture and body proportions as Pelung chickens, however, with a higher weight. Fourteen substitutions were found in the *TYRP1* fragment of F_4 Golden Kamper. The single nucleotide polymorphisms (SNP) had no correlation with the chocolate plumage phenotype in F_4 Golden Kamper. The evaluated SNPs in *TYRP1* were not associated with the brown plumage color phenotype.
Keywords: Chicken, Golden Kamper, Phenotype, Polymorphism, *TYRP1*
[Full text-PDF]

Research Paper

The Effects of Three Commercial Grower Feeds on Performance, Internal Organs, and Carcass traits in Pullet Chickens

Ekeocha AH, Aganga AA, Oluwadele Jf, and Ayoola SK.
J. World Poult. Res. 12(2): 117-123, 2022; pii: S2322455X2200013-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.13

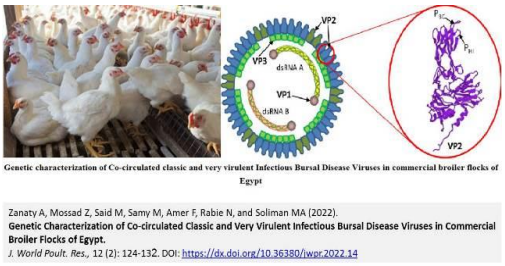
ABSTRACT: Poultry farming is categorized as a developing business venture in most countries, especially Nigeria. This is followed by poultry feed production units ranging from smaller compartments to commercial poultry feed producers. This research study was carried out to examine the physical, and biochemical parameters of feed, growth performance, carcass traits, and visceral organs of pullets fed selected commercial grower feeds and formulated diet. A total number of 1200 *Isa Brown* pullets aged 10 weeks were divided into 4 groups with 5 replicates for each group randomly. This research experiment was completed within 8 weeks. All poultry feeds were filled inside standard polyethylene woven bags in the absence of insects/mold. All poultry feeds, including Top Feed, Chikun Feed, and formulated diet were grouped into mash form except one of the commercial feeds Vital Feed in the pelleted form which is the treatment of the research. There were significant differences in final body weight, weight gain, feed consumed, and feed conversion ratio among the experimental treatments. The least weight was recorded among hens fed Vital feeds with the highest feed intake, which might be due to high fiber content in the feed. The dietary treatment significantly affects the live weight, dressed weight, neck, breast muscle, liver, kidney, gizzard, and abdominal fat of pullet fed different commercial feed and formulated diets. The findings of the current study indicated that a self-formulated diet at the grower stage could replace the commercial poultry feeds used in the study.
Keywords: Body weight, Carcass traits, Grower feed, Pullets, Visceral organs
[Full text-PDF]

Research Paper

Genetic Characterization of Co-circulated Classic and Very Virulent Infectious Bursal Disease Viruses in Commercial Broiler Flocks of Egypt

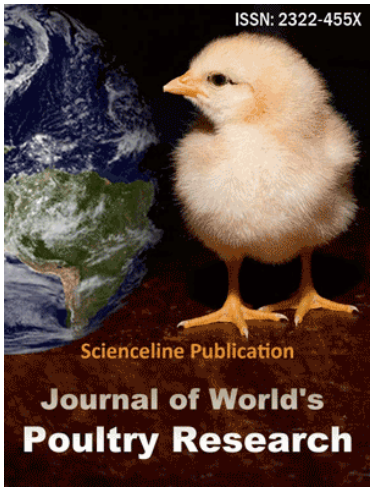
Zanaty A, Mossad Z, Said M, Samy M, Amer F, Rabie N, and Soliman MA.

J. World Poult. Res. 12(2): 124-132, 2022; pii: S2322455X2200014-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.14



ABSTRACT: In recent years, the reintroduction of the infectious bursal disease virus (IBDV), particularly its severe strains, has imposed considerable cost on the Egyptian poultry industry. The goal of the current study was to investigate the molecular features of IBDV in Egypt from June 2019 to April 2021. A total of 30 field samples (bursa of Fabricius) were collected from broiler farms in which the chickens were vaccinated (Transmune 2512 s/c) at hatching. A highly variable region encompassing VP2 gene was targeted for IBDV screening utilizing reverse transcription-polymerase chain reaction (RT-PCR). Of 30 tested samples, 16 were positive by PCR. To isolate the virus, the bursal suspension was injected into 10-11 day embryonated chicken eggs via the chorioallantoic membrane. Five current positive isolates from 2021 were chosen for nucleotide and amino acid (aa) sequence analysis. Phylogenetically, three of the strains under study belonged to the very virulent (vvIBDV) strains, with 97-98% resemblance to Giza 2008 belonging to the (Genogroup 3) IBDV strain. The remaining two strains were identified as a vaccination strain (genotype 1) that matched the winter field 2512 vaccine strain by a similarity percentage of 98. Mutations in the antigenic locations of (P) domain loops were discovered when the sequencing samples were compared to the existing IBD vaccines. The circulating strains were found to be very similar to vvIBDV serotype 1 genotype 3 strains with mutations in the P domain loop providing a potential reason for the circulation of vvIBDV viruses in Egyptian broiler farms despite the vaccination program.
Keywords: Bursa, Classic infectious bursal disease strain, Virulent infectious bursal disease, Virus protein 2 Gene
[Full text-PDF]

Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

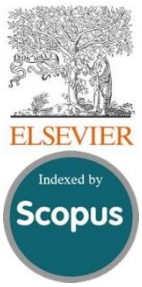
Current Issue: 2022, Vol: 12, Issue: 2 (June 25)

Publisher: SCIENCELINE

The Journal of World's Poultry Research (ISSN: 2322-455X) is an international, peer reviewed open access journal aims to publish the high quality material from poultry scientists' studies to improve domesticated birds production, food quality and safety ... view full aims and scope

www.jwpr.science-line.com

» Indexed/covered by SCOPUS (CiteScore=0.9), NLM Catalog (NLM ID: 101681042), NAAS (Score: 4.79), CIARDRING, Ulrich's™/ ProQuest, PUBDB, ICV 2020= 120.76, TOCs, TIB, WorldCat, EZB, Google Scholar...full index information



» Open access full-text articles is available beginning with Volume 1, Issue 1.

» Digital Archiving: Journal Repository (ePrints)

» Full texts and XML articles are available in ISC-RICeST, Crossref and AGRIS.

» This journal is in compliance with Budapest Open Access Initiative and International Committee of Medical Journal Editors' Recommendations.

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

BOAI 10

» High visibility of articles over the internet.

» This journal encourage the academic institutions in low-income countries to publish high quality scientific results, free of charges... view Review/Decisions/Processing/Policy

Scienceline Publication, Ltd
Editorial Office:
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No:1/6, Yakutiye, Erzurum/25100, Turkey
Homepage: www.science-line.com
Email: administrator@science-line.com
Phone: +90 538-7708824 (Turkey); +1 209-7324265 (Canada)

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 77-84, June 25, 2022

Review Paper, PII: S2322455X2200009-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.9

A Meta-analysis of Fiber Ratio Effects on Growth Performance, Gastrointestinal Traits, and Nutrient Digestibility of Broiler Chickens

Intan Nursiam[1], Muhammad Ridla[2]*, Nahrowi Nahrowi[2], Widya Hermana[2], and Anuraga Jayanegara[2]

[1]*Graduate School of Nutrition and Feed Science, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*
[2]*Departement of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*

*Corresponding author's Email: hmridla@apps.ipb.ac.id

Received: 18 April 2022
Accepted: 04 June 2022

ABSTRACT

Fiber is one of the essential nutrients for broiler chickens. This meta-analysis was carried out to investigate the impacts of fiber fraction ratio on broiler chickens growth performance, digestive characteristics, and nutritional digestibility. The database was compiled from 15 publications reports on the addition of fiber sources in broilers feed. To analyze the effect of acid detergent fiber (ADF) / neutral detergent fiber (NDF) ratio, the mixed model technique was utilized, with ADF/NDF ratio in the feed as a fixed effect and the experiment as a random effect. The ADF/NDF ratio in the feed had no effect on average daily gain, average daily feed intake, and feed per gain ratio in this research. Moreover, a decrease in ADF/NDF ratio in broiler chicken feed increased the relative weight of the gizzard. The relative weight and length of the small intestine and cecum were not affected by the ADF/NDF ratio in the feed. The ADF/NDF ratio in feed enhanced ileal digestibility and total tract apparent retention of most nutrients. The ADF/NDF ratio in the feed had no effect on the jejunal morphology. The minimum ADF/NDF ratio of 0.37 in the feed led to the maximum growth performance, digestive tract development, and optimal nutrient digestibility. In conclusion, controlling the ratio of fiber fraction in broiler chickens feed can improve broiler performance in the non-antibiotic growth promoters era.

Keywords: Broilers chickens, Fiber fraction, Meta-analysis, Performances

INTRODUCTION

The prohibition of antibiotics growth promoters (AGP) in animal feed and antimicrobial resistance has become a global problem over the last three decades. Probiotics, prebiotics, symbiotics, organic acids, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, bacteriophages, clay, and minerals are examples of natural ingredients that can be used to replace AGP in broiler chicken feeds (Gadde et al., 2017; Stefanello et al., 2022). Apart from using a natural AGP alternative, Mateos et al. (2012) proposed that using whole grains, manipulating feed particle size, and increasing fiber in the feed could be effective ways to improve broiler chicken performance in the non-AGP era.

Broiler chickens need fiber to improve the function and development of the digestive system (Mateos et al., 2012). The capacity to promote development in broilers is influenced by the physicochemical characteristics and particle size of the used fiber source. The use of fiber sources in broiler feed has been shown to improve the development of digestive organs, enzyme production, and performance, as well as encouraging the formation of beneficial bacteria (Gonzalez-Alvarado et al., 2007; Jimenez-Moreno et al., 2013a; Sacranie et al., 2012). The use of 2-3% fiber sources in broiler feed can help the growth of the gizzards (Mateos et al., 2012; Shivus, 2011). Fiber can increase the digestibility of amino acids in feed by stimulating the synthesis of hydrochloric acid in the proventriculus, which acts as a precursor for the formation of pepsinogen (Svihus, 2014).

The investigation of methods to improve broiler chicken performance in the era of the AGP ban is still ongoing. Optimizing the development of the digestive tract of broiler chickens by including fiber sources in feed has the potential to improve the performance of broiler

To cite this paper: Nursiam I, Ridla M, Nahrowi N, Hermana W, and Jayanegara A (2022). A Meta-analysis of Fiber Ratio Effects on Growth Performance, Gastrointestinal Traits, and Nutrient Digestibility of Broiler Chickens. *J. World Poult. Res.*, 12 (2): 77-84. DOI: https://dx.doi.org/10.36380/jwpr.2022.9

chickens. The purpose of this study was to determine the effect of fiber fraction ratio in broiler chickens' feed and investigate the effect of using fiber on performance, development of the digestive tracts, and nutrient digestibility of broiler chickens.

MATERIALS AND METHODS

Database development

The database was created based on several types of literature that reported the effects of adding fiber sources on the growth performance of broiler chickens, gastrointestinal properties, and nutrient digestibility. Publication types were found using keywords such as "hull", "fiber", "broiler", and "performance" in Science Direct and Google Scholar. A total of 33 journal papers were included. After checking the suitability of the titles and abstracts, 15 articles were entered into the database (Table 1). The inclusion criteria were the English language of the article, the addition of fiber source, and the measured neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the broiler feed. The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) were followed in this meta-analysis investigation (Moher et al., 2009).

Cellulose, oat hulls, pea hulls, rice hulls, soy hulls, sugar beet pulp, sunflower hulls, wheat bran, and wood were some of the used fiber sources (Table 1). The amount of fiber sources added to the diet varied from 0 (control) to 9%. The assessed variables were growth performance (average daily gain [ADG], average daily feed intake [ADFI], and feed to gain ratio [FG]), gastrointestinal traits (relative organ weight, relative organ length, and pH), and nutrient digestibility (apparent ileal digestibility [AID] and total tract apparent retention [TTAR]).

Data with suitable units of measurement were handled statistically for meta-analysis using a mixed-procedure model (Jayanegara et al., 2019; Hidayat et al., 2021). The PROC MIXED technique was used to execute the analyses in *SAS*® OnDemand for Academics. The ADF/NDF ratio was assigned a fixed impact, whereas the study was assigned a random effect; hence, the analysis contained a random statement. The statistical significance level was set at $p < 0.05$, while the trend level was established at $p = 0.05$-0.10. The ADF/NDF ratio was regarded as a continuous predictor, and the response variables were regressed using the following mathematical model:

$$Y_{ij}=B_0+B_1X_{ij}+s_i+b_iX_{ij}+e_{ij},$$

Where, Y_{ij} is the dependent variable, B_0 denotes the overall intercept across all studies (fixed effect), B_1 refers to the linear regression coefficient of Y on X (fixed effect), X_{ij} signals ADF/NDF ratio as a continuous predictor, s_i stands for the value of research random effect i, b_i is the effect of random research on the regression coefficient of Y on X in research I, and e_{ij} signals the unexplained residual error.

Table 1. Literature included in the meta-analysis of fiber ratio effects on growth performance, gastrointestinal traits, and nutrient digestibility in broiler chickens

Fiber sources	Inclusion	Reference
Oat hulls	0-3%	Barekatain et al. (2017)
Oat hulls, Soy hulls	0-3%	Gonzalez-Alvarado et al. (2007)
Oat hulls, Soy hulls	0-3%	Gonzalez-Alvarado et al. (2008)
Oat hulls, Sugar beet pulp	0-3%	Gonzalez-Alvarado et al. (2010)
Oat hulls, Sugar beet pulp, Cellulose	0-3%	Jimenez-Moreno et al. (2009)
Oat hulls, Sugar beet pulp, Cellulose	0-3%	Jimenez-Moreno et al. (2010)
Pea hulls	0-7.5%	Jimenez-Moreno et al. (2011)
Oat hulls, Sugar beet pulp	0-7.5%	Jimenez-Moreno et al. (2013ab)
Oat hulls, Rice hulls, Sunflower hulls	0-5%	Jimenez-Moreno et al. (2015, 2019)
Wood	0-1%	Monika et al. (2019)
Oat hulls	0-9%	Scholey et al. (2020)
Wheat bran	0-3%	Shang et al. (2020)

RESULTS AND DISCUSSIONS

The effect of the ADF/NDF ratio on broiler chicken performance

Broiler chickens had 40.59±15.97 g/day ADG, 57.38±27.19 g/day ADFI, and 1.38±0.1 FG in this meta-analysis (Table 2). According to the results of the meta-analysis, the ADF/NDF ratio in broilers feed did not have a negative effect on ADG, ADFI, and FG (Table 3). As the ADF/NDF ratio in the feed reveals the proportion of fiber fraction in the broiler feed; the greater value of the ADF/NDF ratio, and the higher fraction of insoluble fiber (cellulose and lignin). Acid detergent fiber consists of cellulose and lignin, which are the two main components of insoluble fiber (Choct, 2009; Choct, 2015a; Choct, 2015b).

Fiber sources are high in insoluble fiber fractions and may resist enzymatic digestion processes in the digestive system and so they cannot be fermented by bacteria in the digestive tract (Mateos et al., 2012). A soluble fiber fraction is a form of fiber that is quickly fermented and has the potential to increase feed viscosity in the digestive system (Sozcu, 2019). Insoluble fiber promotes the development of the upper part of the digestive systems, such as the gizzard, while soluble fiber may be fermented into organic acid, both of which are advantageous to broiler chicken performance (Svihus, 2014; Shang et al., 2020).

Table 2. Descriptive statistics of the studies included in the meta-analysis of fiber ratio effects on growth performance, gastrointestinal traits, and nutrient digestibility in broiler chickens

Parameter		N	Mean	SD	Minimum	Maximum
Performance	ADG (g/bird/day)	49	40.59	15.97	28.60	95.84
	ADFI (g/bird/day)	46	57.38	27.19	39.30	142.40
	FG	46	1.38	0.10	1.25	1.60
Relative organ weight (g/kg BW)	Proventriculus	39	4.73	0.65	3.20	6.10
	Gizzard	47	17.47	5.95	9.50	32.90
	Liver	42	30.02	5.33	21.2	42.3
	Pancreas	14	3.20	0.31	2.70	3.80
	Small intestine	10	47.97	24.61	21.30	77.60
	Caeca	30	5.01	1.79	3.30	10.40
Relative organ length (cm/kg BW)	Small intestine	33	162.78	46.98	78.80	226.00
	Caeca	24	23.52	2.60	20.10	29.20
pH	Proventriculus	27	4.23	0.50	3.37	5.19
	Gizzard	33	3.28	0.58	2.38	4.78
	Duodenum	11	6.13	0.09	5.96	6.23
Apparent Ileal Digestibility (%)	DM	23	71.27	2.31	66.90	75.30
	OM	21	74.76	2.43	70.70	79.30
	CP	23	76.72	3.08	71.30	83.90
	Ash	10	48.65	4.08	42.30	55.60
	Starch	21	94.39	2.36	90.20	98.00
Total Tract Apparent Retention (%)	DM	33	77.59	2.14	73.50	81.60
	OM	33	82.20	2.11	77.80	86.20
	Soluble Ash	33	41.19	6.70	23.40	53.40
	Nitrogen	33	66.90	2.91	61.30	71.60
	EE	33	88.40	3.45	79.80	93.50
AMEn (Kcal)		33	3177.95	80.79	2974.00	3298.46
Jejunal Morphology	Villus Height (µm)	15	929.53	210.27	719.00	1449.00
	Crypt Depth (µm)	15	11.93	21.53	98.00	186.00
	Villus Height/Crypt Depth	15	8.24	1.87	6.72	14.49

N: Number of the sample, SD: Standart Deviation, ADG: Average Daily Gain, ADFI: Average Daily Feed Intake, FG: Feed to Gain ratio, DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, AMEn: Apparent Metabolish Energy.

The effect of the ADF/NDF ratio on the digestive system of broiler chicken

The ADF/NDF ratio in feed affects each digestive organ differently (Table 3). An increase in the ADF/NDF ratio had a negative effect on the proventriculus and gizzard relative weight but had no effect on the liver and pancreas relative weights. According to the model, the ideal ADF/NDF ratio for obtaining the best relative weight of the gizzard is 0.41. According to Svihus (2011), proventriculus produces mucous, hydrochloric acid, pepsinogen, and lipases, on the other hand, the main functions of gizzard include increasing digestibility through feed particle size reduction, mechanical-chemical nutrient degradation of feed ingredients, and regulating the flow rate of feed in the digestive tract.

The physicochemical properties of the fiber source added to the feed are thought to stimulate an increase in the relative weight of the gizzard. According to Jimenez-Moreno et al. (2010), broilers fed oat hulls with particle sizes of 386 µm and 462 µm had relative gizzard weights of 2.73% and 3.3%, respectively. Fiber sources are high in lignin and can linger in the gizzard longer, causing the gizzard muscles to work harder to digest it, thereby stimulating better development of the gizzard (Gonzalez-Alvarado et al., 2008).

The ADF/NDF ratio in the feed had no effect on the relative weight or length of the small intestine. These findings contradict those of Kimiaetalab et al. (2018), who found that the fiber supplementation in broiler feed affects the weight and relative length of the small intestine. Dietary fiber helps the maintenance of small and large intestine integrity by strengthening mucosal structure and functions and increasing the population and diversity of commensal bacteria in the gastrointestinal tract (Jha and Mishra, 2021). Maintaining a balance of soluble and insoluble fiber in the small intestine is of utmost importance; in case there is too much soluble fiber, the viscosity will increase and the flow rate of feed in the small intestine will decrease; the addition of non-starch polysaccharide enzymes is expected to reduce the negative effects of this issue. Broiler chickens need some insoluble fiber for fermentation, in this regard, short-chain fatty acid can be utilized by broiler chickens as an energy source.

The weight and relative length of cecum had no effect on the ADF/NDF ratio in the feed. Through the help of bacteria in the cecum, the cecum aids in water and salt reabsorption as well as the fermentation of uric acid and carbohydrates into ammonia and volatile fatty acid (Svihus et al., 2013a; Svihus et al., 2013b). According to Shang et al. (2020), the addition of 3% wheat bran can enhance the population of *Lachnoclostridium* and *Butyricicoccus*, which can have a role in the production of butyric acid in broiler chicken. The proportion of soluble fiber is directly connected to the ratio of ADF/NDF to cecum function since bacteria in the cecum require a particular quantity of soluble fiber for effective fermentation.

The ratio of ADF/NDF in the feed altered the pH of the proventriculus and gizzard, while the ratio of the fiber fraction in the feed did not affect the pH of the duodenum (Table 3). To create a low pH gizzard, the minimal ADF/NDF ratio in the feed is 0.37. Changes in pH are closely related to the proventriculus and gizzards' increased ability to produce hydrochloric acid, which acts as a precursor for the enzyme pepsinogen (Svihus, 2011) and increases the reflux mechanism between the proventriculus-gizzard and gizzard duodenum resulting in a more optimal level of nutrient digestibility (Hetland et al., 2004).

Jejunal morphology (villus height, crypt depth, and villus height/crypt depth ratio) was unaffected by the ADF/NDF ratio in the feed (Table 3). Monika et al. (2019) reported that increasing the lignocellulose content in the feed causes shortness of jejunal crypt. The use of pea hull as a fiber source up to 7.5% in the feed can minimize the villus height and crypt depth (Jimenez-Moreno et al., 2011). This finding suggests that each organ requires a distinct type of fiber than the others. It is hypothesized that broiler chickens require a suitable composition of soluble fiber, which can function as a prebiotic to support improved intestinal health, in order to produce better jejunal morphology.

The effect of the ADF/NDF ratio on the nutrient digestibility of broiler chickens

The apparent ileal digestibility (AID) of dry matter (DM), organic matter (OM), and Ash, as well as the total tract apparent retention (TTAR) of DM, soluble ash, nitrogen (N), and ether extract (EE), were affected by the ADF/NDF ratio in feed (Table 3). The maximal ADF/NDF ratio values for producing AID DM, OM, and Ash were 0.44, 0.43, and 0.49, respectively, whereas TTAR DM, soluble ash, N, and EE were 0.46, 0.45, 0.44, and 0.51, respectively. The amount of digesta viscosity in the digestive system is related to the fiber ratio in feed. The higher the soluble fiber fraction in the diet, the higher the viscosity, and the lower the amount of nutritional digestibility. Maintaining a balance between the quantity of insoluble fiber and soluble fiber in the feed reduces

viscosity, allowing the feed to be digested more easily (Nursiam et al., 2021).

Apparent metabolizable energy (AMEn) was unaffected by the ADF/NDF ratio in the feed (Table 3). The improved DM and nitrogen digestibility in broilers given more fiber in the feed was strongly tied to proventriculus and gizzard's capacity to produce hydrochloric acid, which functions as a precursor for the enzyme pepsinogen (Svihus, 2011). According to Hetland et al. (2003), adding fiber sources, such as oat hulls can boost bile acid production and amylase enzyme activity. Jimenez-Moreno et al. (2019) reported that the increased levels of fat digestibility in broiler chickens fed oat hulls, sunflower hulls, and rice hulls as a source of fiber in the feed were 89.4%, 89.35%, and 89.9%, respectively, compared to 87% in the control groups.

Table 3. The effect of fiber fraction ratio on growth performance, gastrointestinal traits, and nutrient digestibility in broiler chickens

Respon Parameter	N	Model	Intercept	SE intercept	Slope	SE slope	p-value	RMSE	R^2	AIC
Performance										
ADG (g/bird/day)	49	L	42.168	5.749	4.193	3.332	0.216	3.559	0.994	248
ADFI (g/bird/day)	46	L	61.684	10.028	4.729	3.995	0.245	4.265	0.997	252.4
Feed/Gain Ratio	46	L	1.415	0.042	-0.046	0.072	0.532	0.079	0.918	-135.2
Relative organ weight (g/Kg BW)										
Proventriculus	39	L	5.521	0.407	-1.878	0.802	0.026	0.751	0.825	48.5
Gizzard	47	Q	1.504	7.294	82.846 -100.11	32.851 37.090	0.011	9.512	0.701	260.3
Liver	42	Q	31.799	4.088	-11.678 12.216	16.129 16.822	0.473	3.145	0.954	171.2
Pancreas	14	Q	4.894	1.894	-8.691 10.383	9.916 12.658	0.433	0.524	0.519	-1.2
Small Intestine	10	L	31.163	17.112	21.286	14.034	0.18	8.424	0.985	56.7
Caeca	30	L	5.907	0.936	-1.777	1.14	0.134	0.882	0.968	60.1
Relative organ length (cm/Kg BW)										
Small Intestine	33	Q	151.34	22.592	43.492 -50.411	59.718 71.292	0.486	12.92	0.990	221.5
Caeca	24	L	24.697	2.011	-3.003	3.837	0.444	2.386	0.886	80
pH										
Proventriculus	27	L	3.744	0.374	1.263	0.618	0.054	0.472	0.877	10
Gizzard	33	Q	4.515	1.117	-7.127 9.513	4.994 4.978	0.098	0.883	0.697	38.3
Duodenum	11	Q	5.443	0.735	4.416 -6.255	3.906 4.978	0.249	0.228	0.148	-19.9
Apparent Ileal Digestibility (%)										
DM	23	Q	57.51	6.538	70.029 -79.696	28.3 30.243	0.018	5.253	0.335	86
OM	21	Q	63.664	7.187	58.331 -68.482	31.325 33.575	0.059	5.475	0.322	80
CP	23	Q	71.961	7.326	31.145 -39.25	30.125 31.559	0.232	4.381	0.74	91.2
Ash	10	Q	5.676	23.529	199.76 -204.34	90.326 80.125	0.044	8.45	0.445	32.5
Starch	21	Q	86.428	7.36	39.435 -44.171	31.986 34.23	0.216	5.146	0.364	80.8
Total Tract Apparent Retention (%)										

DM	33	Q	69.812	4.318	35.335	19.612	0.093	3.809	0.574	121.7
					-38.425	21.977				
OM	33	Q	80.205	4.209	9.79	19.104	0.57	3.461	0.64	120.3
					-12.34	21.406				
Soluble Ash	33	Q	5.268	12.901	168.2	58.184	0.009	12.153	0.541	188.2
					-186.54	65.125				
Nitrogen	33	Q	51.585	6.258	74.197	28.739	0.016	6.05	0.411	142.7
					-83.941	32.266				
EE	33	Q	74.383	3.76	59.715	16.434	0.004	3.457	0.856	116.8
					-59.002	18.353				
AMEN (Kcal)	33	Q	2901.79	168.76	1168.02	769.41	0.185	145.682	0.562	341.2
					-1178.38	862.74				
Jejunal Morphology										
Villus Height (µm)	15	L	845.2	269.49	261.8	538.36	0.636	250.816	0.814	167.4
Crypt Depth (µm)	15	L	145.06	34.931	-72.016	81.359	0.395	48.109	0.346	119.8
Villus Height/Crypt Depth	15	Q	-4.269	13.888	64.238	70.027	0.381	4.314	0.3	46.1
					-78.818	86.008				

N: Number of sample, SE: Standard error, RMSE: Root Mean Standard error; AIC: Akaike information criterion, ADG: Average Daily Gain, ADFI: Average Daily Feed Intake, FG: Feed to Gain ratio, DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, AMEn: Apparent Metabolish Energy, L: Linear, Q: Quadratic.

CONCLUSION

In conclusion, broiler chickens require a certain amount of fiber to support optimal growth. The ADF/NDF ratio in the feed should be kept at a minimum of 0.37 to achieve high growth performance, digestive tract development, and optimal nutrient digestibility. Each fiber fraction has a unique impact on the function and growth of the digestive tract. Therefore, it is critical to consider the balance of each fiber fraction in order to promote health, nutritional digestibility, and welfare in broiler chickens.

DECLARATION

Acknowledgments

This research was self-financed and did not receive funding assistance from any party.

Competing interest

The authors declare no conflict of interest.

Authors' contribution

Intan Nursiam contributed to data mining, building a database, data analysis, and preparing the manuscript. Muhammad Ridla, Nahrowi Nahrowi, Widya Hermana, and Anuraga Jayanegara contributed to the design and supervision of the research, the analysis of the results, and the writing of the manuscript. All authors read and approved the final version of the manuscript to publish in the present journal.

Ethical consideration

All authors have checked the ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

REFERENCES

Barekatain R, Swick RA, Toghyani M, and de Koning CT (2017). Interactions of full-fat canola seed, oat hulls as an insoluble fiber source and pellet temperature for nutrient utilization and growth performance of broiler chickens. Poultry Science, 96: 2233-2242. DOI: https://www.doi.org/10.3382/ps/pex008.

Choct M (2009). Managing gut health through nutrition. British Poultry Science, 50: 9-15. DOI: https://www.doi.org/10.1080/00071660802538632.

Choct M (2015a). Feed non-starch polysaccharides for monogastric animals: Classification and function. Animal Production Science, 55: 1360-1366. DOI: https://www.doi.org/10.1071/AN15276.

Choct M (2015b). Fibre-chemistry and function in poultry nutrition. LII Simposio Cientifico de Avicultura, Malaga, Spain. Available at: https://www.wpsa-aeca.es/aeca_imgs_docs/16478_fibra_mingan.pdf.

Gadde U, Kim WH, Oh ST, and Lillehoj HS (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. Animal Health Research

Review, 18(1): 26-45. DOI: https://www.doi.org/10.1017/S1466252316000207.

Gonzalez-Alvarado JM, Jimenez-Moreno E, Gonzalez-Sanchez D, Lazaro R, and Mateos GG (2010). Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive and digestive traits of broilers from 1 to 42 days of age. Animal Feed Science and Technology, 162: 37-46. DOI: https://www.doi.org/10.1016/j.anifeedsci.2010.08.010.

González-Alvarado JM, Jiménez-Moreno E, Lázaro R, and Mateos GG (2007). Effects of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poultry Science, 86: 1705-1715. DOI: https://www.doi.org/10.1093/ps/86.8.1705.

González-Alvarado JM, Jiménez-Moreno E, Valencia DG, Lázaro R, and Mateos GG (2008). Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poultry Science, 87: 1779-1795. DOI: https://www.doi.org/10.3382/ps.2008-00070.

Hetland H, Choct M, and Svihus B (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. Worlds Poultry Science, 60: 415-422. DOI: https://www.doi.org/10.1079/WPS200325.

Hetland H, Svihus B, and Krogdahl A (2003). Effect of oat hull and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. British Poultry Science, 44: 275-282. DOI: https://www.doi.org/10.1080/0007166031000124595.

Hidayat C, Irawan A, Jayanegara A, Sholikin MM, Prihambodo TR, Yanza YR, Wina E, Sadarman S, Krisnan R, and Isbandi I (2021). Effect of dietary tannins on the performance, lymphoid organ weight, and amino acid ileal digestibility of broiler chickens: A meta-analysis. Veterinary World, 14(6): 1405-1411. DOI: https://www.doi.org/10.14202/vetworld.2021.1405-1411.

Jayanegara A, Sujarnoko TUP, Ridla M, Kondo M, and Kreuzer M (2019). Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis. Journal Animal Physiology and Animal Nutrition, 103: 456-465. DOI: https://www.doi.org/10.1111/jpn.13050.

Jha R and Mishra P (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: A review. Journal of Animal Science and Biotechnology, 12: 51. DOI: https://www.doi.org/10.1186/s40104-021-00576-0.

Jimenez-Moreno E, Chamorro S, Frikha M, Safaa HM, Lazaro R, and Mateos GG (2011). Effects of increasing levels of pea hulls in the diet on productive performance, development of the gastrointestinal tract, and nutrient retention of broilers from one to eighteen days of age. Animal Feed Science and Technology, 168: 100-112. DOI: https://www.doi.org/10.1016/j.anifeedsci.2011.03.013.

Jimenez-Moreno E, de Coca-Sinova A, Gonzalez-Alvarado JM, and Mateos GG (2015). Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. Poultry Science, 95(1): 1-12. DOI: https://www.doi.org/10.3382/ps/pev309.

Jimenez-Moreno E, Frikha M, de Coca-Sinova A, Garcia J, and Mateos GG (2013a). Oat hulls and sugar beet pulp in diets for broilers 1. Effects on growth performance and a nutrient digestibility. Animal Feed Science Technology, 182: 33-43. DOI: https://www.doi.org/10.1016/j.anifeedsci.2013.03.011.

Jimenez-Moreno E, Frikha M, de Coca-Sinova A, Garcia J, and Mateos GG (2013b). Oat hulls and sugar beet pulp in diets for broilers 2. Effects on the development of the gastrointestinal tract and on the structure of the jejunal mucosa. Animal Feed Science and Technology, 182: 44-52. DOI: https://www.doi.org/10.1016/j.anifeedsci.2013.03.012.

Jimenez-Moreno E, Gonzalez-Alvarado J M, de Coca-Sinova A, Lazaro R, and Mateos GG (2009). Effect of source of fibre on the development and pH of the gastrointestinal tract of broilers. Animal Feed Science and Technology, 154: 93-101. DOI: https://www.doi.org/10.1016/j.anifeedsci.2009.06.020.

Jimenez-Moreno E, Gonzalez-Alvarado JM, de Coca-Sinova A, Lazaro RP, and Mateos GG (2019). Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 2. Effects on gastrointestinal tract development and nutrient digestibiliy. Poultry Science, 6: 1-17. DOI: https://www.doi.org/10.3382/ps/pey599.

Jimenez-Moreno E, Gonzalez-Alvarado JM, Gonzalez-Sanchez D, Lazaro R, and Mateos GG (2010). Effect of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age. Poultry Science, 89: 2197-2212. DOI: https://www.doi.org/10.3382/ps.2010-00771.

Kimiaeitalab MV, Mirzaie Goudarzi S, Jimenez-Moreno E, Camara L, and Mateos GG (2018). A comparative study on the effects of dietary sunflower hulls on growth performance and digestive tract traits of broilers and pullets fed a pullet diet from 0 to 21 days of age. Animal Feed Science and Technology, 236: 57-67. DOI: https://www.doi.org/10.1016/j.anifeedsci.2017.11.023.

Mateos GG, Jiménez-Moreno E, Serrano MP, and Lázaro R (2012). Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. Journal of Applied Poultry Research, 21:156-174. DOI: https://www.doi.org/10.3382/japr.2011-00477.

Moher D, Liberati A, Tetzlaff J, Altman DG, and Group P (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med, 6(7): e1000097. DOI: https://www.doi.org/10.1371/journal.pmed.1000097.

Monika BIT, Bogucka J, Dankowiakowska A, and Walasik K (2019). Small Intestine morphology and ileal biogenic amines content in broiler chickens feed diets supplemented with lignocellulose. Livestock Science, 241: 104189. DOI: https://www.doi.org/10.1016/j.livsci.2020.104189.

Nursiam I, Ridla M, Hermana W, and Nahrowi N (2021). Effet of fiber source on growth performance and gastrointestinal tract in broiler chickens. IOP Conf. Series: Earth and Environmental Science, 788: 012058. DOI: https://www.doi.org/10.1088/1755-1315/788/1/012058.

Saadatmand N, Toghyani M, and Gheisari A (2019). Effects of dietary fiber and threonine on performance, intestinal morphology and immune responses in broiler chickens.

Animal Nutrition, 5: 248-255. DOI: https://www.doi.org/10.1016/j.aninu.2019.06.001.

Sacranie A, Svihus B, Denstadli V, Moen B, Iji P A, and Choct M (2012). The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poultry Science, 91: 693-700. DOI: https://www.doi.org/10.3382/ps.2011-01790.

Sadeghi A, Toghyani M, and Gheisari A (2015). Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poultry Science, 94: 2734-2743. DOI: https://www.doi.org/10.3382/ps.pev292.

Sadeghi A, Toghyani M, Tabidiyan SA, Foroozandeh AD, and Ghalamkari G (2020). Efficacy of dietary supplemental insoluble fibrous materials in ameliorating adverse effects of coccidial chalange in broiler chickens. Archives of Animal Nututrition, 74: 362-379. DOI: https://www.doi.org/10.1080/1745039X.2020.1764811.

Scholey DV, Marshall A, and Cowan AA (2020). Evaluation of oats with varying hull inclusion in broiler diets up to 35 days. Poultry Science, 99: 2566-2572. DOI: https://www.doi.org/10.1016/j.psj.2019.12.043.

Shang QH, Liu SJ, He TF, Liu HS, Mahfuz S, Ma XK, and Piao XS (2020). Effects of wheat bran in comparison to antibiotics on growth performance, intestinal immunity, barrier function, and microbial composition in broiler chickens. Poultry Science, 99: 4929-4938. DOI: https://www.doi.org/10.1016/j.psj.2020.06.031.

Sozcu A (2019). Growth performance, pH Value of gizzard, hepatic enzyme activity, immunologic indicators, intestinal histomorphology, and cecal microflora of broilers fed diets supplemented with processed lignocellulose. Poultry Science, 98(12): 1-8. DOI: https://www.doi.org/10.3382/ps/pez449.

Stefanello C, Moreira B, Graf WM, Robalo S, Costa ST, Vieira IM, and Miranda DJ (2022). Effects of a proprietary blend of quillaja and yucca on growth performance, nutrient digestibility, and intestinal measurements of broilers. Journal Applied Poultry Research, 31: 100251. DOI: https://www.doi.org/10.1016/j.japr.2022.100251.

Svihus B (2011). The gizzard: Function, influence of diet structure and effects on nutrient availability. Worlds Poultry Science, 67: 207-223. DOI: https://www.doi.org/10.1017/S0043933911000249.

Svihus B (2014). Function of the digestive system. Journal of Applied Poultry Research, 23: 1-9. DOI: https://www.doi.org/10.3382/japr.2014-0.00937.

Svihus B, Choct M, and Classen HL (2013b). Function and nutritional roles of the avian caeca: A review. Worlds' Poulry Science, 69: 249-263. DOI: https://www.doi.org/10.3382/japr.2014.009.

Svihus B, Lund VB, Borjgen B, Bedford MR, and Bakken M (2013a). Effect of intermittent feeding, structural components and phytase on performance and behavior of broiler chickens. British Poultry Science, 54: 222-230. DOI: https://www. .doi.org/10-1080/00071668.2013.772952.

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication
J. World Poult. Res. 12(2): 85-97, June 25, 2022

Research Paper, PII: S2322455X2200010-12
License: CC BY 4.0

DOI: https://dx.doi.org/10.36380/jwpr.2022.10

Effect of Partial Replacement of Yellow Corn by Mango Seed Kernel on Productive Performance, Egg Quality, and Blood Constituents of Laying Hens

Mohamed Elsayed Eid Farag[1], Kout-Elkloub Moustafa Elsayed Moustafa[1], Amina Shaaban El-Saadany[1], and Salma Hashem Abu Hafsa[2]*

[1]*Animal Production Research Institute, Agricultural, Agricultural Research Center, Giza 12619, Egypt*
[2]*Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab, Alexandria 21934, Egypt*

*Corresponding author's Email: hashim_salma@yahoo.com

Received: 09 April 2022
Accepted: 28 May 2022

ABSTRACT

Corn is the main energy source in most poultry feed. Due to rapid climate change, corn production cannot keep up with the demand for food and industrial applications. This necessitated the search for alternatives, such as agro-industrial by-products like mango seed kernel, which is a good source of carbohydrates and protein and can wholly or partly replace corn. The purpose of this study was to investigate the effect of partial replacement of yellow corn (YC) with soaked mango seed kernel (SMSK) on productive and reproductive performance, egg quality, blood biochemistry, hematological parameters, and antioxidants status of local laying hens. A total of 120 local *Gimmizah* breeds (108 females and 12 males) at 32 weeks of age were randomly assigned to four dietary treatments with three replicates (9 hens + 1 male per replicate). The treatments were YC replaced by SMSK at 0, 10, 15, and 20% levels in the hen diets for 12 weeks. Replacing YC with SMSK increased egg production, weight and number of eggs, and egg mass, and improved feed conversion ratio, but feed intake was not affected. Replacement of yellow corn with SMSK did not affect egg quality parameters. The hens in the SMSK 10% and SMSK 15% groups had the highest fertility, hatchability, post-hatch chick weight, and number followed by those in the SMSK 20% group. Groups given varying levels of SMSK had the lowest rate of embryonic mortality. Carcass weight and dressing percentage were positively affected by the 10% and 15% SMSK diet, except for the heart, pancreas, and spleen. Hematological indices were not influenced by dietary SMSK except for higher platelets in the SMSK 20% group. Total protein, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels were similar among treatments. In SMSK groups, serum total cholesterol, triglycerides, and malondialdehyde levels decreased significantly, whereas IgG and catalase levels increased. These findings indicated that SMSK up to 20% could be considered a successful nutritional and health approach and can be partially substituted for YC with no adverse impact on the productive, reproductive and physiological performance of laying hens.

Keywords: Antioxidant status, Egg quality, Egg production, Laying hens, Lipids profile, Mango Seed Kernel, Replacement

INTRODUCTION

The high cost of feed remains the most constraint for poultry production, accounting for about 70-80% of total expenditures in Egypt. Maize as the main energy source in poultry feed accounts for between 50 and 55% of most poultry feed (Vieites et al., 2014). It is used equally in humans' nutrition thus creating intense competition between humans and livestock. This necessitated the search for alternatives like agro-industrial by-products that could wholly or partially replace maize such as mango seed kernel (Kperegbeyi and Onwumere, 2007). Mango (*Mangifera indica Linn*.) is the main fruit crop in Egypt, Egyptian production reached 1.091.535 tons in a harvested area of 265.509 (Hamdy et al., 2021). Since mangoes contain between 9-40% of inedible kernels, a significant amount of kernel waste is generated during industrial processing, causing major disposal issues (Berardini et al., 2005). Therefore, using this massive volume of kernel waste to feed chicken could have an important role in

bridging the problem of scarcity and competition in feed. Mango seed kernel (MSK) has been reported to be a good source of carbohydrates, as well as a high amount of fats that combine to give metabolizable energy (ME) comparable to that of corn (Diarra et al., 2011). Ravindran and Sivakanesan (1996) reported that only 52.5% of MSK carbohydrates were metabolized by poultry. The protein content of dried MSK (6-13%) has a good essential amino acid profile, especially in terms of lysine and methionine comparable to that of corn (Fowomola, 2010; Diarra et al., 2011). Mango seed kernel (MSK) is an alternative because they have appreciable amounts of calcium, potassium, magnesium, unsaturated fatty acids, vitamins C, E, and A, phenolic compounds, and antioxidants (Huber et al., 2012; Kittiphoom, 2012). Moreover, MSK is rich in essential oils, such as stearic and oleic acids that could be fractionated to produce olein and stearin (Gunstone, 2006; Kittiphoom, 2012). It was reported that 50% and 75% of maize can be substituted by boiled MSK in broiler diets without negative effects on blood parameters (Diarra et al., 2011). Although it has a high content of nutrients, the use of MSK in poultry feed is limited due to the presence of anti-nutritional agents, including tannins, cyanogenic glucosides, flavonoids, alkaloids, saponins, and phytates, (Dakare et al., 2012), however, these anti-nutritional agents may not present major constraints to MSK feeding in poultry. Odunsi (2005) found that when maize was replaced with above 10% of raw MSK, the laying rate of hens, egg mass, feed intake, and feed efficiency were decreased, while the internal egg quality was not affected by a substitution ratio of up to 25%. These findings indicated that raw MSK could not easily substitute maize in laying hens' diet. Processing, as soaking, may allow for higher levels of MSK inclusion. However, there was a scarcity of information in Egypt about mango kernel's nutritional potential as a poultry feed. Therefore, this study was carried out to investigate the effects of partial substitution of yellow corn by mango seed kernel on reproductive performance, fertility, hatchability, carcass characteristics, and blood components of layer hens.

MATERIALS AND METHODS

Ethical approval

The present study was performed at El-Sabahia Poultry Research Station, Animal Production Research Institute (APRI), Agricultural Research Center. All the experimental procedures were permitted by the ethics of the Institutional Animal Care and Use Committees of City Scientific Research and Technological Applications (Protocol No. 56-2Y-3022), Alexandria, Egypt. Chickens were cared for using husbandry guidelines derived from El-Sabahia Poultry Research Station standard operating procedures.

Collection and preparation of mango seed kernel

Mango seeds were obtained from a juice factory, Alexandria Governorate, Egypt. To obtain the kernel, mango seeds were cut open with a knife to reveal the kernel, and then diced to minimize particle size. Kernels were soaked with tap water for three days at room temperature to decrease the anti-nutritional factors and air-sundried for three days. Dried kernels were ground in a mill into a powder and kept in an airtight closed bottle until further chemical analysis. The chemical analysis of mango seeds kernel powder as a feed substitute is presented in Table 1.

Table 1. The chemical composition of soaked mango seed kernel

Items	Mango seed kernel
Chemical analysis (Percentage on a dry matter basis)	
Organic matter	97.26
Crude protein	6.42
Crude fiber	3.18
Crude fat	5.66
Nitrogen free extract	82.00
Ash	2.74
Neutral detergent fibre	29.24
Acid detergent fiber	18.32
Acid detergent lignin	5.66
Hemicellulose	10.92
Cellulose	12.66
ME (Kcal/kg diet)	3255.24
Minerals composition (mg/kg)	
Sodium	2985.11
Potassium	7457.63
Calcium	387.27
Magnesium	1688.44
Zinc	38.84

ME: Metabolizable energy

Laying hens, diets, and experimental design

A total of 120 of the local *Gimmizah* breed (108 females + 12 males) at 32 weeks of age were randomly assigned to four treatment groups, with three replicates and each replicate consisted of 9 hens + 1 cock per m^2. The treatments were yellow corn (YC) replaced by soaked MSK at 0, 10, 15, and 20% levels in the hen diets. All birds were housed in a well-ventilated room at an ambient temperature that fluctuated between 28.6°C and 20.3°C

and 55-60% relative humidity with 16 hours of light to 8 hours of darkness under similar managerial and hygienic conditions. The poultry was placed on wheat straw litter at a depth of 5 cm during the experiment period. Hens were fed experimental diets for two weeks (preliminary period) for adaptation. Feed and water were offered *ad libitum* throughout the experiment period of 12 weeks (32 to 44 weeks of age). The basal diet was supplied to meet the nutrient requirements according to the Agriculture Ministry Decree (1996). The calculated analysis of the experimental diets was performed based on Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001) as shown in Table 2.

Table 2. Ingredient of the experimental diets and calculated analysis (percentage on a dry matter basis)

Item	SMSK 0%	SMSK 10%	SMSK 15%	SMSK 20%
Ingredients (%)				
Yellow corn	64.04	57.636	54.434	51.232
Soybean meal (44%)	20.15	19.08	18.13	17.83
Corn gluten (60%)	5.13	6.20	7.15	7.45
Soaked mongo seed kernel	0.0	6.404	9.606	12.808
Vegetable oil	1.2	1.2	1.2	1.2
Salt	0.3	0.3	0.3	0.3
Dicalcium phosphate	1.07	1.07	1.07	1.07
Limestone	7.75	7.75	7.75	7.75
Vitamin and mineral Premix[1]	0.31	0.31	0.31	0.31
DL-methionine	0.05	0.05	0.05	0.05
Calculated analysis[2](%)				
Crude protein	17.32	17.26	17.34	17.32
Ether extract	3.84	3.87	3.90	3.99
Calcium	3.42	3.49	3.51	3.58
Available phosphorus	0.588	0.596	0.601	0.613
Methionine	0.336	0.331	0.327	0.321
Lysine	0.875	0.866	0.861	0.855
ME (Kcal/kg)	2836.45	2837.49	2840.70	2843.63

[1]Three kg of vitamin-mineral premix per ton of feed supplied per kg of diet: Vitamin A 12,000 IU, Vitamin D3 3,000 IU, Vitamin E 40 mg, Vitamin K3 3 mg, Vitamin B1 2 mg, Vitamin B2 6 mg, Vitamin B6 5 mg, Vitamin B12 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg. [2]According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001). SMSK: Soaked mango seed kernel.

Chemical analyses

Samples of the soaked mango seed kernel (SMSK) powder and feed were finely ground through a 1-mm screen in a Cyclotec mill (Cyclotec 1093; Foss, Germany) and stored prior to chemical analysis. Moisture content was determined in dried samples in an oven at 70°C to a constant weight. The content of CP (N 6.25) was determined according to Kjeldahl's Method No. 978.04 (AOAC, 2005). The ether extract (EE) was determined according to the Soxhlet extract method No. 930.09 (AOAC, 2005). The content of ash was determined by Method No. 930.05 (AOAC, 2005). The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to the method of Van Soest et al. (1991). The value of nitrogen-free extract (NFE) was calculated by the difference method. The calculation for nitrogen-free extract is NFE (%) = 100% – (EE % + CP % + Ash % + CF %). The metabolizable energy (ME) content was calculated according to Steel and Torrie (1980) as ME (kcal/kg) = 432 + 27.91 (CP + NFE + 2.25 × EE). Hemicellulose was calculated as Hemicellulose (%) = NDF (%) - ADF (%). Cellulose was calculated using the equation of Cellulose (%) = ADF (%) - ADL (%).

Productive parameters

Individual weights of hens were recorded weekly to determine the final body weight, and body weight change

(final body weight-initial body weight) was calculated. Feed intake was recorded daily and feed conversion ratio (FCR, g of feed/g of egg) was calculated. Egg weight, egg number, and egg production were recorded daily throughout the experiment. Egg mass g/hen/day (egg number × egg weight) was calculated.

Egg quality traits

Egg quality traits were measured monthly using 15 eggs from each treatment group. Exterior and interior egg quality parameters (percentages of albumen, yolk, shell, shell thickness, egg shape index, and Haugh unit) were determined according to Romanoff and Romanoff (1949). The egg yolk visual color scale was determined by matching the yolk with one of the 15 bands by Roche yolk color Fan (Vuilleumier, 1969). Yolk index (YI) was measured according to Funk (1948), and surface area (SA) according to Carter (1970).

Hatchability measurements

Fertility percentage was calculated as the number of fertile eggs/number of eggs set × 100, hatchability percentage was calculated as the number of hatched chicks/ number of fertile eggs × 100, embryonic mortalities, and body weight, and the number of hatched chicks were recorded on the day of hatch.

Carcass traits

At the end of the experiment, three hens per treatment were randomly chosen, individually weighed, and slaughtered. Hens were manually eviscerated, liver, heart, spleen, gizzard, intestinal and caecal weights, and pancreas, abdominal fat, ovary, oviduct, and yellow follicles weights were recorded. Data of carcass traits were expressed as a percentage of live body weight. Intestinal, caecal, and oviduct lengths were measured (cm).

Blood constituents

At the end of the experiment, 5 ml of blood samples collected randomly from 9 hens per treatment from the brachial vein were allocated into sterilized tubes and non-heparinized tubes. The hematological analysis was performed immediately after the collection of the blood. Heparinized blood samples were analyzed for white blood cell counts (WBC), total red blood cell counts (RBC), hemoglobin content (Hb), packed cell volume (PCV) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), the standard deviation in red cell distribution width (RDW-SD), coefficient variation of red cell distribution width (RDW-CV), and platelet count (PLT). Serum in non-heparinized blood tubes was separated by centrifuging at 4°C and 2000 × g for 15 minutes. Thereafter, sera were transferred into Eppendorf tubes and stored at -20°C until further analysis. The following serum metabolites: total protein, albumin, globulin, A/G, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine transferase (ALT), and alkaline phosphatase (ALP) were determined spectrophotometrically using commercial diagnostic kits provided by (Biodiagnostic Co. Giza, Egypt) according to the procedure outlined by the manufacturer. While, globulin was calculated as (total protein-albumin). Catalase and malondialdehyde (MDA) were determined using commercial kits and a spectrophotometer (Shimadzu, Japan) according to the manufacturers' instructions. Immunoglobulins (IgG, and IgM) were determined using kits (Bethyl Laboratories, Montgomery, TX, USA). The ELISA procedure was performed according to the manufacturer's protocol.

Economic efficiency

Economic efficiency was calculated from the input-output analysis which was calculated based on the experimental feed price and egg production during the experiment period. Economic efficiency values were calculated from the price of the experimental diet and eggs produced as net revenue per unit of the total cost of feed consumed. The European Efficiency Factor (EEF) was calculated according to the method described by Nilipour (1998) at the end of the experiment period.

Statistical Analysis

Data were subjected to statistical analyses in a one-way analysis of variance using general linear model procedures of SAS/STAT (Statistical Analysis System, version 9.3, SAS Institute Inc., Cary, NC, USA) (SAS, 2011). The obtained data were tested by an analysis of variance with a one-way design to test the treatment at each sampling, according to the following model:

$$Y_{ij} = \mu + T_i + \mathcal{E}_{ij}$$

Where, y_{ij} denotes the measured value, μ is the overall mean effect, T_i signifies the i^{th} treatment effect, and $\mathcal{E}_{ij}$ refers to the random error associated with the j^{th} hens assigned to the i^{th} treatment. Significant differences among the treatments were determined at $p < 0.05$. All results are presented as least-squares means

RESULTS AND DISCUSSION

Chemical composition of soaked mango seed kernel

The result of the chemical analysis of SMSK content showed organic matter (OM), crude protein (CP), crude fiber (CF), NFE, ash, and ME were 97.26%, 6.42%, 3.18%, 82.0%, 2.74%, and 3255.24 kcal/kg, respectively (Table 1). The composition of the kernel also showed appreciable mineral content. The contents of CP, CF, ash, and NFE in SMSK are in agreement with those of (Odunsi, 2005; Nzikou et al., 2010), which were similar to the current results. The EE content in SMSK was lower than the value reported by Jadhav and Siddiqui (2010), who observed 9% ether extract. The ME value of the current study was in line with (Diarra et al., 2011; Admasu et al., 2020). The chemical composition of SMSK differed from other studies which could be attributable to mango variety, growing environment, handling, and processing methods (Kansci et al., 2008; Diarra et al., 2011; Admasu et al., 2020). The composition of the kernel revealed low levels of protein and fiber but a high content of carbohydrates making it an energy-rich ingredient that could be used to replace corn in poultry feed.

Productive performance

The effect of different levels of SMSK on layers productivity is summarized in Table 3. The results showed that chickens fed a diet containing SMSK at different levels resulted in not only higher egg production ($p < 0.05$), but also a significant increase in egg weight, egg number, and egg mass ($p < 0.05$), compared to the control group. Feed intake was not significantly differed between the treatment groups ($p > 0.05$), meanwhile, the FCR was better for hens given different levels of SMSK compared to those fed the control diet ($p < 0.05$). Furthermore, hens in the SMSK 10% group had the highest ($p < 0.05$) final body weight and body weight change, however, these weights decreased in the SMSK 20% group, with no differences between the layers on 15% SMSK and control groups.

Table 3. Effect of replacing corn with soaked mango seed kernel on productive performance of Gimmizah laying hens at 44 weeks of age

Parameters	Treatments				P value
	SMSK 0%	SMSK 10%	SMSK 15%	SMSK 20%	
Egg production (%)	0.66 ± 0.02^{b}	0.74 ± 0.01^{a}	0.71 ± 0.02^{a}	0.72 ± 0.03^{a}	0.033
Egg weight (g)	51.77 ± 0.31^{b}	52.99 ± 0.44^{a}	53.29 ± 0.47^{a}	52.91 ± 0.26^{a}	0.028
Egg number	71.42 ± 2.15^{b}	$80.58 \pm 0.4.03^{a}$	76.98 ± 2.78^{a}	77.71 ± 3.92^{a}	0.015
Egg mass (g/hen/d)	35.22 ± 1.24^{b}	39.50 ± 2.12^{a}	37.94 ± 1.07^{a}	38.07 ± 2.01^{a}	0.021
Feed intake (g/h/d)	130.33 ± 1.01	128.17 ± 3.74	131.34 ± 2.43	129.56 ± 1.46	0.737
FCR (g feed/ g egg)	2.52 ± 0.02^{a}	2.42 ± 0.05^{b}	2.46 ± 0.02^{b}	2.45 ± 0.03^{b}	0.009
Initial body weight (g)	1840.50 ± 44.06	1828.1 ± 38.83	1838.0 ± 45.51	1811.5 ± 39.04	0.846
Final body weight (g)	1997.67 ± 10.74^{b}	2066.4 ± 11.49^{a}	2007.6 ± 10.66^{b}	1980.41 ± 7.35^{c}	0.016
Body weight change (g)	157.17 ± 9.88^{b}	238.29 ± 11.51^{a}	169.61 ± 13.06^{b}	98.91 ± 14.79^{c}	0.022

$^{a-c}$ different superscripts in the same row differed significantly at $p < 0.05$. SMSK: Soaked mango seed kernel, FCR: Feed conversion ratio

Concerning the productive performance criteria, MSK can be recycled after anti-nutritional factors were removed and might be considered a potentially valuable source of antioxidants and employed as a feed ingredient (Ajila et al., 2007; El Boushy and van der Poel, 2000). Mango seed kernels were soaked and air-dried before being ground into a powder and blended with the ingredients of the experimental diets in this study to avoid the adverse effects of anti-nutritional factors in MSK. The results showed that replacing YC with 20% SMSK in hen diets improved egg production, egg weight and number, egg mass, and FCR ($p < 0.05$) without having a significant influence on feed intake ($p > 0.05$). The present results are inconsistent with Odunsi (2005) who observed a decrease in egg production rate, egg mass, feed consumption, feed efficiency, and increased weight loss in hens when maize was replaced with more than 10% raw MSK. While the result in body weight change in the SMSK 20% group agrees with Odunsi (2005). The results of the present study revealed that replacing YC with SMSK in the diet of layer hens had no effect on feed intake demonstrating its acceptability. The current result agrees with Beyene et al. (2019) reported that replacing maize grain with MSK had no effect on dry matter intake, however, the present result

disagrees with the result of the same author that FCR and egg production were unaffected. The present result is inconsistent with Diarra et al. (2010) who found no significant differences in feed intake between the control group and the group fed 40% boiled MSK replacement by maize, however, the FCR was significantly improved when 60% of maize was replaced. As reported by Diarra et al. (2011), processing the mango kernel using a method such as boiling reduces the tannin content in the kernel. A certain minimum daily feed intake is necessary to satiate the poultry's appetite and to allow the digestive tract to function properly, which may indicate the normal palatability of MSK diets. These findings suggest that SMSK could be an effective corn substitute for improving digestion and preventing digestive disorders in the hens' gut. Consequently, SMSK, which is a non-competitive and non-conventional feedstuff, was found to be useful as an economic substitution for energy materials such as corn for animals (Omer et al., 2019). Generally, the current result indicated that replacing YC with SMSK in laying hens' diets improved the feed conversion ratio, whereas decreased the final body weight and the change in body weight. The present result agrees with those of Kumar et al. (2010) who reported improved FCR with increased MSK doses in the diet of broilers. Beyene et al. (2019) found that body weight change did not differ among treatments when fed on MSK. However, Admasu et al. (2020) reported that replacing maize with boiled MSK at 40% in the diet of broilers did not affect FCR, but improved final body weight. Govindappa et al. (2022) revealed no significant difference in body weight, feed intake, feed efficiency, and survivability between control and treatment groups when Giriraja birds were fed with graded levels of MSK powder. Previous studies showed that the inclusion of some residues/ wastes (apple, banana, chicory, cabbage, citrus, grape, mango, pineapple, peas, and pumpkin) in the diets of poultry and livestock significantly improved animal reproductive traits (Nkosi et al., 2016; Alnaimy et al., 2017).

Egg quality

No significant effect was observed due to partial substitution of dietary YC with SMSK on all egg quality traits ($p > 0.05$, Table 4). The findings of this study are consistent with the report of Odunsi (2005) who reported that the internal egg quality characteristics were not affected by a substitution rate of up to 25%. Beyene et al. (2019) reported no significant effect on the substitution of maize with MSK on albumen and yolk weight and height, and Haugh unit, shell quality parameters. There was no difference among treatments in eggshell weight and thickness which could be explained by the calcium levels of the treatment diets laid within the recommended range. Albumin quality is not significantly affected by feeding, but the decrease in Haugh unit is affected by the age of the hen and the egg storage conditions (Williams, 1992). Thus, the absence of difference in these parameters between the treatments indicated that replacing YC with MSK up to 20% does not affect the Haug unit. The albumen height determines the Haugh unit of the egg. The greater the albumen height, the greater the Haugh unit and the better egg quality. But in this study, albumen height did not differ significantly between treatments ($p > 0.05$). Haugh unit referenced was within the recommended range of 70-100, indicating good quality of the eggs (Lewko and Ornowicz, 2009). Yolk index values were within the acceptable range 0.33-0.50 for fresh eggs (Ihekoronye and Ngoddy, 1985). Odunsi (2005) found no differences in yolk weight and height, color, and yolk index between hens fed on SMSK and control diets.

Table 4. Effect of replacing corn with soaked mango seed kernel on egg quality traits of Gimmizah laying hens

Parameters	Treatments				p value
	SMSK 0%	SMSK 10%	SMSK 15%	SMSK 20%	
Egg shape index (%)	77.06 ± 0.73	76.44 ± 0.65	76.88 ± 0.71	76.74 ± 0.67	0.851
Egg shell weight (%)	5.81 ± 0.46	5.75 ± 0.39	5.77 ± 0.42	5.75 ± 0.41	0.687
Shell thickness (mm)	0.39 ± 0.05	0.38 ± 0.03	0.38 ± 0.04	0.38 ± 0.03	0.773
Haugh unit (%)	92.59 ± 0.56	92.70 ± 0.58	92.93 ± 0.56	93.05 ± 0.66	0.884
Yolk weight (%)	17.23 ± 1.06	17.21 ± 1.31	17.23 ± 1.52	17.14 ± 0.99	0.891
Yolk index (%)	4.32 ± 0.12	4.31 ± 0.09	4.30 ± 0.13	4.26 ± 0.10	0.793
Yolk color score	8.07 ± 0.67	7.93 ± 0.59	7.82 ± 0.53	7.85 ± 0.61	0.659
Albumen weight (%)	31.18 ± 2.41	30.85 ± 2.19	31.09 ± 2.27	30.94 ± 2.33	0.699

SMSK : Soaked mango seed kernel

Fertility and hatchability

The results of fertility and hatchability traits are presented in Table 5. Hens in the SMSK treatment groups had the highest fertility and hatchability percentages, followed by the SMSK 20% group ($p < 0.05$). There were significant differences in embryonic mortality between the treatment groups at all development phases ($p < 0.05$). Groups of hens given varying levels of SMSK had the lowest rate of embryonic mortality in the early, middle, and late phases of development compared with those of the control group. The pip embryo's weight was unaffected. The number and weight of post-hatched chicks produced by hens were highest in the SMSK 10% and SMSK 15% groups, followed by those produced by the SMSK 20% group ($p < 0.05$). These results disagree with those of Beyene et al. (2015) who found that replacing maize with MSK up to 100% did not indicate significant differences in fertility, hatchability, and quality of hatched chicks. There is scarce information on the effect of MSK on egg fertility, hatchability, and hatched chick weight in laying poultry. As documented by Hocking et al. (2002) who reported that insufficient nutrients in breeders' diets led to poor hatchability rate. On the other hand, the contents of crude protein and energy of the treatment diets were effective on egg fertility and hatchability which was confirmed by the present results. Tona et al. (2004) reported that there is a positive correlation between egg weight and hatching chick weight. Similarly, the present result showed that the weight of hatched chicks was significantly increased when hens were fed a diet containing SMSK up to 20%, and this result could be attributed to the improvement of egg weight by replacing the yellow corn with 20% SMSK in the diets. Several studies have reported that a diet rich in protein and sufficient energy has been shown to improve egg weight and hatching chick weight (Gunawardana et al., 2008; King'ori et al., 2010; Shim et al., 2013). There was a significant decrease in embryonic mortality at all phases of development between the SMSK treatment groups ($p < 0.05$). The crude protein and energy contents of treatment diets were comparable; thus, no nutritionally related embryonic mortality was expected. Similarly, Hocking et al. (2002) observed that hens fed a high-protein diet had a decreased embryonic mortality rate than hens fed a low-protein diet.

Carcass characteristics

The two groups SMSK 10% and SMSK 15% had the highest carcass weight, dressing percentage, and relative weight of intestine, proventriculus, ovary, Oviduct, and the number of yellow follicles, as well as had the longest length of intestine, caecum, and oviduct, while the SMSK20% group had the lowest values compared with the control group (Table 6). The relative weight of the heart, pancreas, and spleen did not differ between SMSK and control groups. Hens in the SMSK 20% group had the lowest relative weight of caecum and gizzard and abdominal fat but had the highest relative weight of liver compared with the other SMSK and control groups. The present findings are consistent with those of Odunsi (2005) who observed increased liver weight in broilers fed raw MSK at 20% as a maize substitute compared with the control diet, whereas heart and spleen weights were not affected by the substitution. Diarra (2015) reported that replacing maize with cooked MSK up to 25% had no adverse effect on liver and heart weights. The increased liver weight on the raw MSK diet could be a response to toxic substances that were overcome through processing, as the liver is primarily concerned with detoxification by converting toxic substances into easily excreted forms (Diarra, 2015).

Table 5. Effect of replacing corn with soaked mango seed kernel on hatchability traits, embryonic mortality, and chick weight of Gimmizah laying hens

Parameters	Treatments				p value
	SMSK 0%	SMSK 10%	SMSK 15%	SMSK 20%	
Fertility (%)	95.98 ± 0.84^{b}	97.91 ± 0.78^{a}	96.80 ± 0.71^{ab}	97.50 ± 0.79^{a}	0.034
Hatchability (%)	90.74 ± 0.24^{c}	94.61 ± 0.43^{a}	94.60 ± 0.44^{a}	91.64 ± 0.37^{b}	0.001
Embryonic mortality phases (%)					
Early	2.00 ± 0.27^{a}	1.33 ± 0.19^{b}	1.11 ± 0.20^{b}	1.11 ± 0.18^{b}	0.026
Middle	1.00 ± 0.26^{a}	0.89 ± 0.25^{ab}	0.67 ± 0.24^{b}	0.44 ± 0.17^{b}	0.033
Late	3.33 ± 0.49^{a}	1.67 ± 0.36^{b}	2.11 ± 0.48^{b}	1.56 ± 0.35^{b}	0.016
Pip	0.78 ± 0.22	0.56 ± 0.18	0.68 ± 0.20	0.78 ± 0.24	0.828
Post-hatch chick quality					
Hatched chicks (Number)	69.67 ± 1.02^{c}	74.11 ± 0.91^{a}	73.26 ± 0.88^{a}	71.48 ± 0.99^{b}	0.001
Hatched chicks weight (g)	33.06 ± 0.57^{c}	36.96 ± 0.83^{a}	36.90 ± 0.69^{a}	34.01 ± 0.47^{b}	0.012

$^{a-c}$ different superscripts in the same row differed significantly at $p < 0.05$. SMSK: Soaked mango seed kernel.

In this study, the highest value of the liver weight of the SMSK 20% group was within the normal range for layer hens, therefore hepatotoxicity related to SMSK feeding of 20% was not expected. Abdullahi (2012) found no negative effects of substituting 100% of dietary maize with SMSK on liver and spleen weights of broilers, but heart weight increased with increasing SMSK levels in the diet. However, Amao and Siyanbola (2013) observed lower liver, heart, and pancreas weights when fed broilers 30% dry heat-treated MSK substitute for maize and compared with the control. Diarra et al. (2010) found that MSK treatment had no significant influence on any of the carcass yields or abdominal fat. They also reported that a boiled MSK diet may substitute up to 60% of maize in broiler diets with no deleterious impacts on growth, carcass parameters, or health.

Table 6. Effect of replacing corn with soaked mango seed kernel on carcass traits of Gimmizah laying hens at 44 weeks of age

Parameters	Treatments				p value
	SMSK 0%	SMSK 10 %	SMSK 15%	SMSK 20%	
Live body weight (g)	1728.33 ± 110.53	1831.67 ± 109.72	1806.67 ± 106.89	1738.33 ± 103.58	0.647
Carcass (g)	1076.67 ± 0.18^{b}	1179.00 ± 0.31^{a}	1166.67 ± 0.25^{a}	1036.33 ± 0.10^{c}	0.006
Dressing (%)	62.47 ± 0.23^{b}	64.49 ± 0.28^{a}	64.76 ± 0.30^{a}	59.59 ± 0.27^{c}	0.001
Intestine weight (%)	96.67 ± 0.94^{b}	103.07 ± 1.84^{a}	102.33 ± 1.93^{a}	85.67 ± 1.85^{c}	0.001
Intestine length, (cm)	174.16 ± 0.99^{b}	181.67 ± 1.16^{a}	181.33 ± 1.13^{a}	167.00 ± 0.96^{c}	0.001
Caecum weight (%)	22.69 ± 0.42^{a}	22.33 ± 0.37^{a}	22.67 ± 0.35^{a}	20.15 ± 0.30^{b}	0.018
Caecum length, (cm)	24.05 ± 1.03^{b}	26.33 ± 1.36^{a}	27.33 ± 1.41^{a}	21.33 ± 0.80^{c}	0.012
Gizzard (%)	27.66 ± 2.71^{ab}	32.33 ± 2.41^{a}	30.07 ± 2.29^{a}	25.67 ± 2.71^{b}	0.025
Proventriculus (%)	9.68 ± 0.22^{b}	10.83 ± 0.29^{a}	11.07 ± 0.24^{a}	8.83 ± 0.25^{c}	0.001
Liver (%)	45.83 ± 0.31^{b}	45.90 ± 0.37^{b}	45.93 ± 0.26^{b}	47.25 ± 0.46^{a}	0.015
Heart (%)	8.37 ± 0.84	9.03 ± 0.69	8.93 ± 0.77	8.83 ± 0.70	0.744
Pancreas (%)	5.28 ± 0.66	4.74 ± 0.59	5.33 ± 0.45	4.84 ± 0.54	0.683
Spleen (%)	3.33 ± 0.31	3.27 ± 0.24	3.08 ± 0.20	3.33 ± 0.28	0.867
Abdominal fat (%)	80.68 ± 2.22^{a}	74.61 ± 1.89^{b}	72.93 ± 2.71^{b}	56.33 ± 1.02^{c}	0.011
Ovary (%)	7.54 ± 0.23^{b}	9.03 ± 0.31^{a}	8.80 ± 0.27^{a}	6.37 ± 0.19^{c}	0.007
Yellow follicles (%)	58.00 ± 2.97^{a}	60.67 ± 3.02^{a}	57.67 ± 2.91^{a}	45.33 ± 2.62^{b}	0.028
Yellow follicles number	6.33 ± 0.39^{b}	7.00 ± 0.48^{a}	7.00 ± 0.48^{a}	5.00 ± 0.41^{c}	0.016
Oviduct (%)	55.33 ± 3.15^{b}	65.33 ± 3.26^{a}	62.33 ± 2.98^{a}	51.00 ± 2.37^{c}	0.031
Oviduct length (cm)	61.73 ± 1.33^{b}	67.69 ± 1.12^{a}	66.67 ± 1.26^{a}	52.44 ± 0.73^{c}	0.001

$^{a-c}$ different superscripts in the same row differed significantly at $p < 0.05$. SMSK: soaked mango seed kernel.

Blood hematological, biochemical, and immunological parameters

The results in Table 7 indicate the influence of SMSK on blood hematological, biochemical and immunological parameters for layer hens. There were no significant differences in the values of WBCs, RBCs, lymphocytes, neutrophils, monocyte, eosinophil, hemoglobin, MCH, RDW-CV, and RDW-SD between treatment groups. The SMSK 10% group had significantly lower PCV and MCV values, however, the SMSK 0% and 20% groups had greater platelets than the other treatment groups, with no significant differences between the SMSK 0% and SMSK 20% groups or between the SMSK 10% and SMSK 15% groups ($p < 0.05$). A significant increase in MCHC value was observed in the SMSK 10% group compared to the other groups ($p < 0.05$). The addition of SMSK at different levels in *Gimmizah* hens' diet did not affect the blood-related biochemical traits such as total protein, AST, ALT, and ALP (Table 7).

The results revealed a significant ($p < 0.05$) decrease in albumin, and albumin/ globulin ratio in SMSK 15% and SMSK 20% groups, with no significant differences between SMSK10% and SMSK 0% groups or between SMSK 15% and SMSK 20% groups. Hens fed with different levels of SMSK had the lowest ($p < 0.05$) values of total cholesterol and triglycerides, with no significant

differences between the SMSK groups. The results of serum immunoglobulin, IgG, and IgM for *Gimmizah* hens are shown in Table 7. The IgG concentration was significantly increased with increasing SMSK in the hen diet, whereas the addition of SMSK in the hen diet had no effect on the concentration of IgM ($p < 0.05$). Although the SMSK groups had significantly higher serum catalase concentration than the control group, however, the SMSK 0% group had significantly greater MDA concentration than the SMSK groups ($p < 0.05$).

Table 7. Effect of replacing corn with soaked mango seed kernel on hematological, biochemical, and immunological parameters of Gimmizah laying hens

Parameters	Treatments				p value
	SMSK 0%	SMSK 10 %	SMSK 15%	SMSK 20%	
Blood hematology					
White blood cell $\times10^3/\mu L$	12.34 ± 1.65	12.89 ± 1.41	12.12 ± 1.49	12.43 ± 1.33	0.927
Lymphocyte (%)	67.67 ± 6.75	69.33 ± 6.93	65.08 ± 6.08	71.67 ± 7.12	0.682
Neutrophils (%)	23.67 ± 6.73	23.33 ± 4.42	26.67 ± 7.33	23.67 ± 6.84	0.904
Monocyte (%)	5.33 ± 0.62	5.66 ± 0.66	5.00 ± 0.55	5.03 ± 0.53	0.467
Eosinophil (%)	3.42 ± 0.35	3.07 ± 0.21	3.37 ± 0.29	3.67 ± 0.39	0.624
Red blood cell $\times10^6/\mu L$	3.29 ± 0.43	3.41 ± 0.37	3.52 ± 0.51	3.49 ± 0.49	0.672
Platelets	11.00 ± 1.24^a	7.00 ± 1.21^b	7.67 ± 0.96^b	10.00 ± 1.19^a	0.032
Haemoglobin (g/dl)	10.54 ± 0.30	10.61 ± 0.45	10.69 ± 0.47	11.03 ± 0.42	0.441
PCV (%)	38.9 ± 2.16 ^{a}	34.4 ± 1.88^b	39.3 ± 2.21^a	40.3 ± 2.27^a	0.036
MCV (fL/cell)	11.82 ± 0.2^a	10.09 ± 0.18^c	11.16 ± 0.26^b	11.26 ± 0.21^b	0.002
MCH (pg)	3.20 ± 0.19	3.11 ± 0.14	3.04 ± 0.11	3.16 ± 0.16	0.814
MCHC (g/dl)	27.09 ± 1.11^b	30.84 ± 1.12^a	27.20 ± 0.98^b	27.37 ± 1.03^b	0.016
RDW-CV	11.57 ± 1.47	11.77 ± 1.61	10.20 ± 1.88	12.00 ± 1.76	0.525
RDW-SD	25.37 ± 2.89	25.93 ± 2.54	27.53 ± 2.66	28.27 ± 2.94	0.633
Blood biochemical parameters					
Total protein (g/dl)	3.47 ± 0.28	3.36 ± 0.22	3.27 ± 0.25	3.21 ± 0.29	0.774
Albumin (g/dl)	1.61 ± 0.19^a	1.44 ± 0.27^{ab}	1.29 ± 0.22^b	1.13 ± 0.31^b	0.021
Globulin (g/dl)	1.86 ± 0.06^b	1.92 ± 0.12^{ab}	1.98 ± 0.11^a	2.08 ± 0.14^a	0.032
Albumin/Globulin ratio	0.87 ± 0.13^a	0.75 ± 0.12^{ab}	0.65 ± 0.11^b	0.54 ± 0.14^b	0.029
Total cholesterol (mg/dl)	195.67 ± 5.06^a	85.33 ± 8.63^b	76.00 ± 14.66^b	59.33 ± 26.84^b	0.007
Triglyceride (mg/dl)	251.67 ± 31.65^a	112.60 ± 11.64^b	99.46 ± 9.46^b	94.67 ± 22.53^b	0.002
AST (U/L)	24.06 ± 3.07	25.33 ± 2.66	27.27 ± 3.16	25.62 ± 2.37	0.698
ALT (U/L)	11.67 ± 2.15	13.07 ± 2.22	12.43 ± 1.85	13.37 ± 2.16	0.837
ALP (U/L)	851.33 ± 137.62	751.67 ± 116.94	735.33 ± 125.75	719.67 ± 135.71	0.547
Immunological parameters					
IgG (mg/dl)	39.47 ± 1.13^c	42.80 ± 1.28^b	43.63 ± 1.31^{ab}	44.97 ± 1.41^a	0.016
IgM (mg/dl)	11.67 ± 1.52	10.49 ± 1.37	11.13 ± 1.33	10.32 ± 1.47	0.673
Antioxidant status					
Catalase (U/L)	25.94 ± 4.65^b	35.86 ± 4.11^a	36.22 ± 3.74 ^{a}	39.97 ± 5.05^a	0.009
MDA (mmol/l)	2.69 ± 0.13^a	2.01 ± 0.19^b	1.94 ± 0.21^b	1.76 ± 0.27^b	0.008

$^{a-c}$ different superscripts in the same row differed significantly at $p < 0.05$. SMSK: Soaked mango seed kernel, PCV: Packed cell volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, RDW-SD: Standard deviation in red cell distribution width, RDW-CV: Coefficient variation of red cell distribution width, AST: Aspartate aminotransferase, ALT: Alanine transferase, ALP: Alkaline phosphatase, MDA: Malondialdehyde.

The impact of dietary treatments on the host was evident in hematological indices. The results of the present study agree with Soomro et al. (2013) who found that utilizing mangchico pulp as a supplement at rates of 2, 3, and 4% /kg had no effect on hemoglobin, RBCs, and WBCs counts. Similarly, Odunsi (2005) reported that replacing MSK with maize at 0, 5, 10, 15, 20, and 25% in broiler feed had no effect on lymphocyte count. However, Moustafa et al. (2019) stated that replacing 10, 15, and 20% of corn with MSK in the cockerels' diets had a significant impact on hemoglobin, PCV%, RBC_S, WBC_S, lymphocytes, and neutrophils counts. Odunsi (2005) found that replacing maize with 10% MSK significantly increased the hemoglobin content of broilers. Amao and Siyanbola (2013) reported that substituting maize with dry heat-treated MSK up to 20% in broiler feed increased hemoglobin concentration. The present findings on most blood hematological parameters were comparable indicating that the immune status of hens fed SMSK substitute YC in the diet can be attributed to the presence of various phenolic compounds in MSK treatment diets, which have an antioxidant effect (Ahmed, 2014) and cause the health-promoting properties of MSK, including analgesic, antioxidant, anti-microbial, and anti-inflammatory activities, which were confirmed by previous results obtained by other researchers (Cojocaru et al., 1986; Anila and Vijayalakshmi, 2003; Garrido et al., 2004).

The findings of this study agree in part with those of Moustafa et al. (2019) who reported that cockerels fed 10% MSK had the highest total protein and globulin levels, but that there were no significant differences between the MSK 20% and control groups. Odunsi (2005) found that replacing MSK with maize by 10% in the diet of broilers increased total protein and globulin levels. However, Amao and Siyanbola (2013) found that the total protein level was significantly high in broilers fed 10% heat-treated MSK. In terms of total cholesterol and triglycerides, our findings are consistent with those of Moustafa et al. (2019) who observed that increasing MSK levels as a substitute for corn in a significant reduction in plasma cholesterol when compared with the control group. Zhang et al. (2017) reported that dietary 0.28% mango saponin supplementation reduced plasma total cholesterol levels in cockerels. This could be explained by the presence of mangiferin in mango saponin. The hypocholesterolemia effect of MSK may be related to containing components of flavonoids that may inhibit lipid peroxidation that regulates cholesterol synthesis.

More so, the determinants of proper liver function; ALT, AST, and ALP demonstrated that the liver is normal and healthy among the treatments since ALT, AST, and ALP liver function test values do not differ significantly among treatments ($p > 0.05$). This is also an indicator of the nutritional adequacy of all diets because of the blood response to feeding. Abang et al. (2018) showed that serum ALT of quails given sun-dried MSK diet treatments was within the normal range but Serum AST of quails fed 25% sun-dried mango kernel meal was below the lower limit among the treatments.

In the current study, the improvement in immune response and antioxidant status may be attributed to the fact that MSK contains various phenolic compounds that are considered antioxidant agents (Cojocaru et al., 1986). Moustafa et al. (2019) reported that the use of MSK at 10% and 15% replacement of corn caused a significant increase in IgG and IgM values compared with the control group, whereas, no significant differences were observed between 20% SMSK and control groups. All examined blood parameters were equivalent to those reported as normal for poultry in the literature (Faniyi, 2002). The fact that blood parameters between the treatments are similar to their normal values implies that all diets are nutritionally adequate. Since the blood profile provides a helpful investigation and demonstrative tool in nutritional assessment and health implications, there is evidence to further suggest that dietary tannin levels were below the thresholds as described by Smith (2001). Hematology and biochemistry in the broilers were not affected when dietary maize was replaced with 100% soaked MSK (Abdullahi, 2012), 60% boiled MSK (Diarra et al., 2010), and 30% dry heat-treated MSK (Amao and Siyanbola, 2013). These findings suggested that when considering MSK as an ingredient of poultry feed, a variety of criteria such as species, age, processing method, and environmental conditions need to be considered.

Economic efficiency

The economic efficiency of using SMSK as a partial substitute for yellow corn for laying hens was shown in Table 8. The SMSK utilized as a corn substitute demonstrated higher economic efficiency than the control group. The relative economic efficiency of corn substituted with 20% SMSK rose by about 28% compared with the control group. Similarly, Moustafa et al. (2019) reported that the feed cost/kg weight gain of cockerels was lower in the group fed 10% MSK as a yellow corn substitute.

Table 8. Economic efficiency of Gimmizah laying hens fed yellow corn and soaked mango seed kernel diets

Parameters	Treatments			
	SMSK 0%	SMSK 10%	SMSK 15%	SMSK 20%
EEF	228.78	266.97	255.15	233.28
Egg number/hen	178.56	201.44	192.44	194.27
Price/ egg (L.E)	1.50	1.50	1.50	1.50
Total price of eggs /hen (L.E)	267.84	302.16	288.66	291.41
Feed intake (g/h/d)	130.33	128.17	131.34	129.56
Total feed intake/hen (kg)	11.73	11.54	11.82	11.66
Price/ ton feed (L.E)	6085.485	5801.178	5680.397	5526.141
Total feed cost/hen (L.E)	71.38	66.94	67.14	64.43
Net revenue/hen (L.E)	196.46	235.22	221.52	226.98
Economic efficiency	2.75	3.51	3.30	3.52
Relative economic efficiency (%)	100	127.64	120.00	128.00

SMSK: Soaked mango seed kernel. EEF: European Efficiency Factor. Price/ egg (L.E.), according to the local market price at the experimental time. Total price of eggs /hen (L.E.) = egg number/ hen × Price/ egg (L.E.). Daily feed intake (g). Total feed intake/hen, kg = (FI (g/hen/day) /1000) X 30 days (Experiment period, days). Price/ Kg feed (L.E.), based on the average price of diets during the experimental time. Total feed cost/hen (L.E.) = total feed intake/hen, kg × price/ Kg feed (L.E.). Net revenue/hen (L.E.) = total price of eggs /hen (L.E.) - total feed cost/hen (L.E.). Economic efficiency = net revenue / hen (L.E.)/ total feed cost/ hen (L.E.). L.E: Egyptian pound

CONCLUSION

Based on this study, soaked mango seed kernel (SMSK) up to 15% can be considered a successful nutritional and health approach and can be replaced partially by yellow corn in the laying hen diet without any deleterious impact on productivity, fertility, hatchability, quality of hatched chicks, or physiological performance. In terms of economic efficiency, SMSK at different levels outperformed the control group. These findings encourage further research on the application of SMSK as a substitute for yellow corn in poultry feed.

DECLARATIONS

Authors' contribution

Farag, Moustafa, El-Saadany, and Abu Hafsa created the idea and designed the study. Farag and Abu Hafsa collected data. Abu Hafsa wrote the paper and performed the statistical analysis. Abu Hafsa drafted the manuscript and approved the final manuscript. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

Competing interests

The authors declared that they have no competing interests.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Consent to publish

All authors informed their consent prior to inclusion in the study.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors before the submission. The final results of the statistical analysis have been also checked and confirmed by all authors.

REFERENCES

Abang FB, Nwani OJ, and Shittu HA (2018). Serum biochemical composition of laying Japanese quails (*coturnix coturnix japonica*) fed diets containing sun-dried mango (*Mangifera* spp) kernel meal (SMKM). European Journal of Agriculture and Forestry Research, 6: 19-23. Available at: https://www.eajournals.org/journals/european-journal-of-agriculture-and-forestry-research-ejafr/vol-6-issue-3-june-2018/serum-biochemical-composition-of-laying-japanese-quails-coturnix-coturnix-japonica-fed-diets-containing-sun-dried-mango-mangifera-spp-kernel-meal-smkm/

Abdullahi I (2012). Nutritive value of differently processed mango seed kernel (local variety (kanbiri) and mixed samples) meal in broiler diets. M.Sc. Thesis, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria, p. 85. DOI: http://www.doi.org/10.13140/RG.2.2.33745.79206

Abdullahi I, Omage J J, Idachaba CU, and Abbas L M (2020). Performance of broiler finisher chickens fed varied levels of mango seed kernel meal as replacement for maize. Nigerian Journal of Animal Production 44: 209-214. DOI: http://www.doi.org/10.51791/njap.v44i1.780

Admasu S, Wondifraw Z, and Gash M (2020). Effects of replacing maize with boiled mango (*Mangifera* indica) seed kernel on feed intake, body weight gain and feed conversion ratio of Cobb 500 broiler

chicken. Poultry, Fisheries and Wildlife Sciences, 8: 211. DOI: https://www.doi.org/10.35248/2375-446X.20.8.211

Agriculture Ministry Decree (1996). The standard properties for ingredients, feed additives and feed manufactured for animal and poultry. El-Wakaee El-Masria, Amirria Press Cairo, Egypt, No. 192, p. 95.

Ahmed MT (2014). Effect of Mangifera indica L. (Mango) kernel on Clarias Gariepinus (African Catfish) fingerlings infected with Aeromonas caviae. M.V. Sc. Thesis, Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. Available at: https://www.semanticscholar.org/paper/EFFECT-OF-MANGIFERA-INDICA-L.-(MANGO)-KERNEL-ON-Ahmad/d6ce6c24afc4cb2138ae149cbfec6c1a103cef94

Ajila CM, Bhat SG, and Rao UP (2007). Valuable components of raw and ripe peels from two Indian mangovarieties. Food Chemistry, 102: 1006-1011. DOI: http://www.doi.org/10.1016/j.foodchem.2006.06.036

Alnaimy A, Gad AE, Mustafa MM, Atta MAA, and Basuony HAM (2017). Using of citrus byproducts in farm animals feeding. Open Access Journal of Science, 1: 1-11. DOI: http://www.doi.org/10.15406/oajs.2017.01.00014

Amao EA, and Siyanbola MF (2013). Carcass and physiological response of broilers fed dry heat-treated mango (*Mangifera* indica) kernel-based diet. Global Journal of Poultry Farming and Vaccination, 1: 59-63. DOI: http://www.doi.org/10.5897/IJLP12.011

Anila L, and Vijayalakshmi NR (2003). Antioxidant action of flavonoids from Mangifera indica and Emblic Officinalis in hypercholesterolemic rats. Food Chem. 83: 569-574. DOI: http://www.doi.org/10.1016/S0308-8146(03)00155-9

Association of Official Analytical Chemists (AOAC) (2005). Official methods of analysis, 18th edition, AOAC International: Rockville, MD, USA.

Berardini N, Knodler M, Schieber A, and Carle R (2005). Utilization of mango peels as a source of pectin and polyphenolics. Innovative Food Science and Emerging Technologies, 6: 442-452. DOI: http://www.doi.org/10.1016/j.ifset.2005.06.004

Beyene G, Niguse M, Berhe A, and Arefaine D (2019). Boiled mango (mangifera indica) seed kernel could replace maize grain in layer's diet in Northern Ethiopia. Journal of the Drylands, 9: 986-995.

Beyene G, Niguse M, Gebreslasie L, and Berhe A (2015). Evaluation of replacing maize with mango (*Mangifera indica*) kernel on fertility and hatchability parameters of layer hens. Livestock Research for Rural Development, 27: 241. Available at: http://www.lrrd.org/lrrd27/12/beye27241.htm

Carter TC (1970). Why do egg shells crack?. World's Poultry Science Journal, 26: 549-561. DOI: http://www.doi.org/10.1079/WPS1970001

Cojocaru M, Droby S, Glotter E, Goldman A, Gottlieb HE, and Jacoby B (1986). 5-(12-heptadecenyl)- resorcinol, the major component of the antifungal activity in the peel of mango fruit. Phytochemistry, 25: 1093-1095. DOI: http://www.doi.org/10.1016/S0031-9422(00)81560-5

Dakare MA, Danladi AA, Abel SA, and Sunday EA (2012). Effects of processing techniques on the nutritional and antinutritional contents of mango (*Mangifera* indica) seed kernel. World Journal of Young Researchers, 2: 55-59. Available at: http://www.academia.edu/3131051/Effects_of_Processing_Techniques_on_the_Nutritional_and_Antinutritional_Contents_of_Mango_Mangifera_indica_Seed_Kernel

Diarra SS (2015). Potential of mango (*Mangifera* indica L.) seed kernel as a feed ingredient for poultry: A review. World's Poultry Science Journal, 70: 279-288. DOI: https://www.doi.org/10.1017/S0043933914000294

Diarra SS, Saleh B, Kwari ID, and Igwebuike JU (2011). Evaluation of boiled mango kernel meal as energy source by broiler chickens in the semi-arid zone of Nigeria. International Journal of Science and Nature, 2: 270-274. Available at: https://www.semanticscholar.org/paper/EVALUATION-OF-BOILED-MANGO-KERNEL-MEAL-AS-ENERGY-BY-Igwebuike/37afb90381cf9d864eb44d547bc53924bbdef014

Diarra SS, Usman BA, and Igwebuike JU (2010). Replacement value of boiled mango kernel meal for maize in broiler finisher diets. Journal of Agricultural and Biological Science, 5: 47-52.

El Boushy ARY, and van der Poel AFB (2000). Handbook of poultry feed from waste: Processing and use. Springer-Verlag New York, p. 428 Available at: https://www.feedipedia.org/node/5403

Faniyi GF (2002). Inclusion of discarded cashew kernels in the diets of cockerel chicks. In: Fanimo, A.O. and Olanite, J.A. (editors). Contributory role of animal production in national development. Proceedings 7th Annual Conference of Animal Science Association of Nigeria. September 16-19. University of Agriculture, Abeokuta, Nigeria, pp. 109-111. Available at: https://www.ajol.info/index.php/tjas/article/view/31084

Feed Composition Tables for Animal and Poultry Feedstuffs Used in Egypt (2001). Technical Bulletin No.1, the central lab for feed and food, Ministry of Agric., Egypt. Available at: http://wvj.science-line.com/attachments/article/60/WVJ%2010(1)%2088-92,%20March%2025,%202020%20Feed%20Additives%20for%20Functional%20Eggs%20in%20Fayoumi%20Hens.pdf

Fowomola MA (2010). Some nutrients and antinutrients contents of mango (*Mangifera* indica) seed. African Journal of Food Science, 4: 472-476. Available at: http://www.academicjournals.org/app/webroot/article/article1380729035_Fowomola.pdf

Funk EM (1948). The relation of the yolk index determined in natural position to the yolk index as determined after separating the yolk from the albumen. Poultry Science, 27: 367. DOI: http://www.doi.org/10.3382/ps.0270367

Garrido G, Gonzalez D, and Lemus Y (2004). *In vivo* and *in vitro* antiinflammatory activity of *Mangifera* indica L. extract (Vimang). Pharmacological Research, 50: 143-149. DOI: http://www.doi.org/10.1016/j.phrs.2003.12.003

Govindappa P, Indresh HC, and Jayanaik (2022). Effect of replacement of maize with graded levels of mango seed kernel powder on growth performance in Giriraja bird. The Pharma Innovation Journal, SP-11: 558-561. Available at: https://www.thepharmajournal.com/special-issue?year=2022&vol=11&issue=1S&ArticleId=10184

Gunawardana P, Roland Sr DA, and Bryant MM (2008). Effect of dietary energy and protein on performance, egg composition, egg solids, egg quality and profits in molted Hy-Line W36 Hens during phase 2. International Journal of Poultry Science, 6: 739-744. DOI: http://www.doi.org/10.3923/ijps.2007.739.744

Gunstone FD (2006). Minor specialty oils. In: Fereidoon Shahidi, Nutraceutical and specialty lipids and their co-products. CRC Taylor and Francis, pp. 91-126. Available at: http://www.crcpress.com/product/isbn/9781574444995

Hamdy AE, Khalifa SM, and Abdel-Aziz HF (2021). Early removal of mango inflorescences increase tree production and fruit quality (Mangifera indica L. cv. 'Tommy Atkins'). Arab Universities Journal Agricultural Sciences, Ain Shams Univ, Cairo, Egypt, 29: 409-418. DOI: http://www.doi.org/10.21608/ajs.2021.42130.1252

Hocking PM, Maxwell MH, Robertson GW, and Mitchell MA (2002). Welfare assessment of broiler breeders that are food restricted after peak rate of lay. British Poultry Science, 43: 5-15. DOI: http://www.doi.org/10.1080/00071660120109818

Huber K, Queiroz JH, Moreira AVB, and Ribeiro SMR (2012). Caracterização química do resíduo agroindustrial da manga Ubá (*Mangifera indica* L.): uma perspectiva para a obtenção de

antioxidantes naturais. Revista Brasileira de Tecnologia Agroindustrial, Curitiba. 6: 640-652. DOI: http://www.doi.org/10.3895/S19813686202000100003

Ihekoronye AI and Ngoddy PO (1985). Integrated Food Science and Technology for the Tropics. Macmillan, London, UK, p. 386. Available at: https://agris.fao.org/agris-search/search.do?recordID=US201300674821

Jadhav NV and Siddiqui MF (2010). Handbook of poultry production and management, 2nd edition, Jaypee Brothers Medical Publishers, New Delhi, India, 383. Available at: http://www.worldcat.org/title/handbook-of-poultry-production-and management/oclc/727063435

Kansci G, koubala BB, and Mbome IL (2008). Biochemical and physicochemical properties of four mango varieties and some quality characteristics of their jams. Journal of Food Process Preservation, 32: 644-655. DOI: http://www.doi.org/10.1111/j.1745-4549.2008.00204.x

King'ori AM, Tuitoek JK, Muiruri HK, and Wachira AM (2010). Effect of dietary crude protein levels on egg production, hatchability and post-hatch offspring performance of Indigenous chickens. International Journal of Poultry Science, 9: 324-329. DOI: http://www.doi.org/10.3923/ijps.2010.324.329

Kittiphoom S (2012). Utilization of mango seed. International Food Research Journal, 19: 1325-1335. Available at: http://ifrj.upm.edu.my/19%20(04)%202012/5%20IFRJ%2019%20(04)%202012%20Kittiporn%20(375).pdf

Kperegbeyi JI, and Onwumere OS (2007). Effect of raw and toasted Bambara groundnut (*Vigna subterranean* (L.) verdcourt) on performance of growing cockerels. In: Book of Abstract of the 32nd Annual conference, NSAP, held at the University of Calabar, Calabar, Nigeria, 32: 188-191. Available at: https://www.semanticscholar.org/paper/EFFECT-OF-RAW-AND-TOASTED-BAMBARA-GROUNDNUT-(-VIGNA-Ironkwe-Esonu/24ba68d0491d66fdd5d32219c1b44359a1766ac8

Kumar D, Moni C, and Singh P (2010). Effects of feeding MSK on performance, carcass characteristics and cost of feeding in broiler starter. Indian Journal of Poultry Science, 45: 46-49.

Lewko L and Ornowicz EG (2009). Egg albumen quality as affected by bird origin. Journal of Central European Agriculture, 10: 399-409. Available at: https://jcea.agr.hr/en/issues/article/776

Moustafa, Kout-Elkloub, M. EL.; Breakaa, M. A.; El-Saadany, A. S., and Farag, M. E. (2019). Effect of dietary mango seed kernel (mangifera indica) as partial replacement of corn on productive and physiological performance of growing gimmizah cockerels. Egyptian Poultry Science, 39: 865-879. DOI: http://www.doi.org/10.21608/EPSJ.2019.67507

Nkosi BD, Meeske R, Ratsaka MM, Langa T, Motiang MD, and Groenewald IB (2016). Effects of dietary inclusion of discarded cabbage (Brassica oleracea var. capitata) on the growth performance of South African Dorper Lambs. South African Journal of Animal Science, 46: 1-7. DOI: http://www.doi.org/10.4314/sajas.v46i1.5

Nzikou JM, Kimbonguila A, Matos L, Loumouamou B, Pambou-Tobi NP, Ndangui CB, Abena AA, Silou T, Scher J, and Desobry S (2010). Extraction and characteristics of mango seed kernel oil. Research Journal Environmental and Earth Sciences, 2: 31-35. Available at: http://www.maxwellsci.com/print/rjees/v2-31-35.pdf

Odunsi AA (2005). Response of laying hens and growing broilers to the dietary inclusion of mango (*Mangifera* indica L.) seed kernel meal. Tropical Animal Health and Production, 37: 139-150. DOI: http://www.doi.org/10.1023/B:TROP.0000048455.96694.85

Omer HAA, Tawila MA, Gad SM, and AbdelMagid SS (2019). Mango (*Mangifera* indica) seed kernels as untraditional source of energy in Rahmani sheep rations. Bulletin of the National Research Centre, 43: 176. DOI: http://www.doi.org/10.1186/s42269-019-0241-4

Ravindran V and Sivakanesan R (1996). The nutritive value of mango seed kernels for starting chicks. Journal of the Science of Food and Agriculture, 71: 245-250. DOI: http://www.doi.org/10.1002/(SICI)1097-0010(199606)71:2<245::AID-JSFA578>3.0.CO;2-C

Romanoff AL and Romanoff AS (1949). The avian egg. John Wiley and Sons, New York. 109: p 414. DOI: http://www.doi.org/10.1126/science.109.2834.414.a

SAS (Statistical Analysis System Software) (2011). Statistical Analysis System Software, Version 9.3. Edition; SAS/STAT. Program SAS Inst. Inc.: Cary, NC, USA.

Shim MY, Song E, Billard L, Aggrey SE, Pesti GM, and Sodsee P (2013). Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poultry Science, 92: 2687-2696. DOI: http://www.doi.org/10.3382/ps.2012-02569

Smith AJ (2001). Poultry: The tropical agriculturalist. Revised edition. Macmillan Education, Ltd. London and Oxford. Available at: https://www.amazon.com/Poultry-tropical-agriculturalist-Anthony-Smith/dp/0333791495

Soomro H, Rind MI, Sanjrani SN, Magsi AS, Barham GS, Pirzada SA, and Sahito HA (2013). Effect of partial mango pulp mixing in ration on behavior and production of broiler. International Journal Plant Animal Science, 1: 30-36. Available at: https://internationalscholarsjournals.org/journal/ijpas/articles/effect-of-partial-mango-pulp-mixing-in-ration-on-behaviour-and-production-of-broiler

Steel RGD and Torrie JH (1980). Principles and procedures of statistics, a biometrical approach. 2nd Ed. McGraw Hills Book Co., New York, U.S.A. Available at: https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=383208

Tona K, Onagbesan O, De Ketelaere B, Decuypere E, and Bruggemen V (2004). Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight and chick posthatch growth to forty-two days. Journal Applied Poultry Research, 13: 10-18. DOI: http://www.doi.org/10.1093/japr/13.1.10

Van Soest PJ, Robertson JB, and Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74: 3583-3597. DOI: http://www.doi.org/10.3168/jds.S0022-0302(91)78551-2

Vieites FM, Nalon RP, Santos AL, Branco PAC, Souza CS, Nunes RV, Calderano AA, and Arruda NV (2014). Performance, carcass and noble cuts yield of broilers fed diets supplemented with *Solanum glaucophyllum*, Londrina. 35: 1617-1626. DOI: http://www.doi.org/10.5433/1679-0359.2014v35n3p1617

Vuilleumier JP (1969). The "Roche yolk color fan" an instrument for measuring yolk color. In: P., Hunton (editor). World Animal Science, Poultry production. Elsevier, Tokyo, pp. 457-481. DOI: http://www.doi.org/10.3382/PS.0480767

Williams KC (1992). Some factors affecting albumen quality with particular reference to Haugh unit score. World Poultry Science Journal, 48: 5-16. DOI: http://www.doi.org/10.1079/WPS19920002

Zhang YN, Wang J, Qi B, Wu SG, Chen HR, Luo HY, Yin DJ, Lü FJ, Zhang HJ, and Qi GH (2017). Evaluation of mango saponin in broilers: Effects on growth performance, carcass quality, meat quality, and plasma biochemical indices. Asian Australasian Journal of Animal Science, 30: 1143-1149. DOI: https://www.doi.org/10.5713/ajas.16.0847

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 98-106, June 25, 2022

Research Paper, PII: S2322455X2200011-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.11

Serological and Molecular Detection of Local Infectious Bronchitis Virus in Vaccinated Broiler Chickens in Diyala Governorate, Iraq

Karrar A Jasim, Amer K Al-Azzawi*, Talib J Kadhim, and Karim S AL-Ajeeli

Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq

* Corresponding author's E-mail: alikianee@gmail.com

Received: 18 April 2022
Accepted: 03 June 2022

ABSTRACT

The infectious bronchitis virus (IBV) is one of the most important *Coronaviridae* viruses, infecting the upper respiratory tract of chickens and leading to considerable losses in the poultry industry across the globe. Many outbreaks have recently occurred among IBV-vaccinated chicken farms in the Diyala Governorate of Iraq resulting in significant economic losses. As a result, the purpose of the present study was to investigate whether IBV can be a source of infection spread in IBV-vaccinated commercial broiler flock farms in Diyala Governorate. In this regard, ELISA was used as a serological test and RT-PCR as a molecular detection technique. Serum samples were collected from chickens suspected of IBV at 16 and 23 days of age. The results showed a significant increase of IgG antibodies in such serum samples at days 16 and 23 of age indicating the infections of the broilers with IBV. However, at the age of 2-3 weeks, the samples of kidney, liver, trachea, and lungs were collected from clinically and sub-clinically infected flocks, and also postmortem samples were sampled from all farms. Two sets of previously reported primers were created for this purpose in the S1-protein gene region. According to the findings of the present investigation, IBV was found in 83% of samples. Finally, despite immunization with IB4/91, IBV was prevalent in broiler chicken farms in the study area confirmed by serology and molecular biology tests. This finding indicates the possibility of genetic difference between the locally discovered IBV and the administered IBV vaccine. A study on the production of local vaccines can be useful in controlling IBV infections.

Keywords: ELISA, Infectious bronchitis viruses, RT-PCR

INTRODUCTION

Infectious bronchitis (IB) is one of the most common contagious respiratory disorders in poultry that affects chickens of all types, ages, sexes, and breeds. The *Coronavirinae* subfamily of the *Coronaviridae* family includes the infectious bronchitis virus. The IBV genome is a single-stranded, positive-sense RNA virus from the gammacoronavirus genus (Cavanagh and Gelb, 2008).

The first infectious bronchitis virus produced by the Mass serotype was discovered in North Dakota, USA, and spread around the world since then (Schalk and Hawn, 1931). Over the years and decades, hundreds of additional IBV genome variations emerged as a result of genetic instability during the propagation or development of the virus (Jordan, 2017). Although chickens are the most common natural hosts for IBV infection, other avian species, such as pigeons, geese, peafowl, pheasants, and ducks can also disseminate IBV albeit clinical indications appear to be limited (Liu et al., 2005; Awad et al., 2014). Infectious bronchitis virus infection in poultry has a short incubation period (24-48 hours) and spreads horizontally by aerosol (coughing and sneezing) and contaminates chicken litter, feed, drinking water, equipment, or other fomites, infecting the poultry flocks in a short time (Chhabra et al., 2015).

Although there has never been a record of IBV transmission via vertical circulation within the embryo, the virus can be transferred from infected hens by contaminating the shell of hatching eggs through shedding from the alimentary tract or the oviduct (Boltz et al., 2004; Saif et al., 2008). Hens of all ages are vulnerable to the IBV, which can lead to great economic losses, although very young chicks have demonstrated more significant respiratory distress, as well as higher morbidity and death,

To cite this paper: Jasim KA, Al-Azzawi AK, Kadhim TJ, and AL-Ajeeli KS (2022). Serological and Molecular Detection of Local Infectious Bronchitis Virus in Vaccinated Broiler Chickens in Diyala Governorate, Iraq. *J. World Poult. Res.*, 12 (2): 98-106. DOI: https://dx.doi.org/10.36380/jwpr.2022.11

as compared to older chickens (Britton and Cavanagh, 2007; Cavanagh and Gelb, 2008).

Initially, it was thought that all IB viruses belonged to a single prototype known as Massachusetts (Mass), which had been detected in commercial chicken flocks (Cavanagh and Naqi, 2003). Several IBV strains have been discovered and isolated from layer and broiler farms in Iraq, Iran, and Egypt, and the virus is considered a dominant strain (Liu et al., 2006; Mahmood et al., 2011; Kahya et al., 2013). Many outbreaks of clinically recognized IBV infections have been observed in chicken farms in the Diyala Governorate of Iraq despite the vaccination program of the private sector to suppress the disease.

The poultry business in Diyala Governorate suffers from economic losses as a result of these epidemics. Accordingly, the goal of this study was to examine the IBV molecular assay in selected broiler chicken flocks in the Diyala governorate using RT-PCR and show the status of disease outbreaks in vaccinated farms.

MATERIALS AND METHODS

Ethical approval

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (134); September 2020, K, A, T, and K).

Design of the study

From September 2020 to June 2021, many outbreaks of clinically identified IBV occurred in broiler chicken farms in the privet sections of Diyala Governorate. Clinical signs included gasping, dyspnea, and nasal discharge in these broiler chickens. The mortality rates varied from 30% to 50%. The tracheal bifurcation was blocked with fibrinonecrotic cast in deceased chickens, as well as pericarditis and perihepatitis due to co-infections. There were also swollen kidneys with inflated tubules (Figure 1).

Sample collection

Blood samples

For blood samples, 2 ml of the blood was collected aseptically from the wing vein of the clinically infected chickens aged 16-23 days using disposable syringes (Terumo, Japan). All collected blood samples were allowed to remain for 1 hour for clotting at room temperature. In the next step, the serum was collected, placed in a sterile Eppendorf tube, labeled, and then frozen at -30°C for antibody evaluation against IBV by ELISA test.

Tissue samples

The samples were collected from kidney, liver, trachea, and lungs of infected chickens (5 samples from each farm). The postmortem samples of the mentioned tissues were also collected from each farm too (5 samples from each farm, Figure 1). These samples were processed later for molecular detection of the IBV.

ELISA test

The Zoetis IBV ELISA kit was used to track IBV antibody levels in infected flocks. All ELISA monitoring studies were conducted by Zoetis IBV Ab, United States using the synbiotic developed Profile 2.0 Windows computer program (ELISA – Synbiotics Corporation, United States). The ELISA kits were used according to the manufacturer's protocol using an automated microplate reader (ELx800, BIO-TEK Instruments Inc., USA). The antibody titer in each sample was quantified using the software provided by the manufacturer. The geometric mean titer for each group of serum samples was also calculated using the same software. Processing of serum samples for the ELISA test was followed according to the instruction manual of the above-mentioned ELISA kit manufacturer.

Molecular detection

The reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect IBV at the molecular level. Accordingly, postmortem tissue samples of the kidney, liver, trachea, and lung (n=10) from clinically and sub-clinically infected broiler chickens aged 2-3 weeks were collected as necropsy findings and were processed for RT-PCR (Figure 1).

Viral RNA was isolated from tissue samples of the kidney, liver, trachea, and lung by the Quick-RNATM Viral Kit (France) following the instruction provided by the manufacturer. In this technique, the first step was the conversion of RNA to cDNA, and the second was to amplify the DNA template using a customized program employing PrimeScriptTM RT reagent Kit intending to execute reverse transcription optimized for RNA to cDNA conversion (O'Connell, 2002).

The RT-PCR reaction volume was expected to be 25 µl (23.5 µl and 1.5 µl as template). All PCR components were vortexed together and centrifuged quickly to remove any remaining liquid from the tubes' sides. A new reaction was made in ice, gently mixed and vortexed for 4 seconds,

and then centrifuged shortly. A programmed thermal cycler was used to transfer the RT-PCR tubes (Eppendorf, USA, O'Connell, 2002).

Using the commercially available kit Maxime TM PCR PreMix Kit (i-Taq^{TM}), the following RT-PCR condition was used to amplify the IBV S1 gene in a single-tube experiment with a final reaction volume of 25µl. As previously stated by the company of Integrated DNA Technologies firm, Canada, the oligonucleotide primers were utilized in the detection of IBV (Jones et al., 2005). Two pairs of primers were used which included (SX1 and SX2) and (SX3 and SX4). The first PCR amplification (SX1 and SX2) and the second nested PCR (SX3 and SX4) were used to generate a copy DNA of 393 base paris region of S1 gene (Table 1).

Amplification was performed using a 35-cycles protocol that included denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes (O'Connell, 2002).

The amplicons were electrophoresed on a 1.5% (w/v) agarose gel in 1XTBE buffer, stained with Red safe Nucleic acid staining, and flanked by a 100 bp ladder as a molecular weight marker purchased from Intron (Korea) following the study conducted by Jones et al. (2005).

Statistical analysis

Statistical analysis was run in SPSS program version 24. The Chi-square test was chosen to indicate the differences between the samples. The significant differences were indicated by the Duncan test at the level of $p \leq 0.05$ (Steel and Torrie, 1980).

Figure 1. Broiler chickens infected with infectious bronchitis virus showed dyspnea with abdominal breathing (gasping, **A**), tracheal bifurcation showed completed obstruction with fibrinonecrotic cast (arrow, **B**), Pericarditis and perihepatitis (arrow) due to co-infections across with IBV (**C**), and swollen kidneys with distended tubules (**D**).

Table 1. Oligonucleotide primers used in the detection of IBV as previously described

Primer Name	Sequence(5′-3`)	Company	Product Size (bp)
SX1F SX2R	5'-TCCACCTCTATAAACACCYTTAC - 3′ 5'-TCCACCTCTATAAACACCYTTAC - 3′	Integrated DNA Technologies Canada).	393bp
SX3F SX4R	5'-TAATACTGGYAATTTTTCAGATGG - 3′ 5'-AATACAGATTGCTTACAACCACC - 3′	Integrated DNA Technologies Canada).	393bp

RESULTS

ELISA test results

ELISA was performed using serum diluent at a single dilution (1:50). All tests included both positive and negative controls. The ELISA test was used to detect specific antibodies to the IBV infection.

The interpretation of ELISA results was done according to the manufacturer's instruction of the kit supplied information (ProFlok TM IBV, Zoetis, USA). The results of IBV titer represented a comparison between IBV antibody level of field serum samples and the positive samples of control sera. In case of the Coefficient of Variance (CV) is greater than 30%, it means a very early exposure of the flock to IBV or the flocks have a bimodal population or a non-uniform flock. According to the manufacturer guidelines of the kit, chickens with ELISA titers below the detectable level throughout the testing period were considered negative (titer < 600, OD = 0.529, and S/P ratio = 0.150). In all sampled fields, sera were collected for detection of the antibodies twice (days 16 and 23 of age).

The antibody titers of chickens aged 16 days in Baqoubah, Kanaan, Baladroze, Almoqdadia, Alkhalis, and Alwajehia groups were found to be 1044.2778 ± 249.95382, 761.4444 ± 182.60843, 2380.3889 ± 317.56748, 1190.8333 ± 200.12109 1373.4444 ± 183.23802 and 728.6111 ± 101.57859 respectively.

In comparison to the day 16, field samples had considerably greater levels of anti-IBV IgG ELISA titer on 23 days of age, the mean titers (11488.153 ± 2376.1111 for Baqubah, 14008.657 ± 3103.2312 for Kanan, 12527.342 ± 6164.3434 for Baladroze, 15709.5563 ± 3589.3356 for Almoqdadia, 11328.6732 ± 3436.5900 for Alkhalis, and 13744.6754 ± 1305.6578 for Alwajehia) showed significant differences among different farms ($p \leq 0.05$) and revealed that all flocks were considered infected according to the instructions of the manual kit of ELISA in six different regions of Diyala Governorate as showed in (Table 2 and figures 2 and 3).

Table 2. Titers of antibodies of IBV (n= 10) at different ages and different regions of Diyala Governorate, Iraq, in broiler chickens (Mean ± standard error)

Regions	16 days	23 days
Baqubah	1044.2778 ± 249.95382 $^{BC\,b}$	11488.153 ± 2376.1111 $^{C\,a}$
Kanaan	761.4444 ± 182.60843 $^{C\,b}$	14008.657 ± 3103.2312 $^{B\,a}$
Baladroze	2380.3889 ± 317.56748 $^{A\,b}$	12527.342 ± 6164.3434 $^{C\,a}$
Almoqdadia	1190.8333 ± 200.12109 $^{BC\,b}$	15709.5563 ± 3589.3356 $^{A\,a}$
Alkhalis	1373.4444 ± 183.23802 $^{B\,b}$	11328.6732 ± 3436.5900 $^{C\,a}$
Alwajehia	728.6111 ± 101.57859 $^{C\,b}$	13744.6754 ± 1305.6578 $^{B\,a}$

^{abc}: Different superscripts in a row means significant ($p \leq 0.05$). ^{ABC}: Different superscripts in a column mean significant ($p \leq 0.05$).

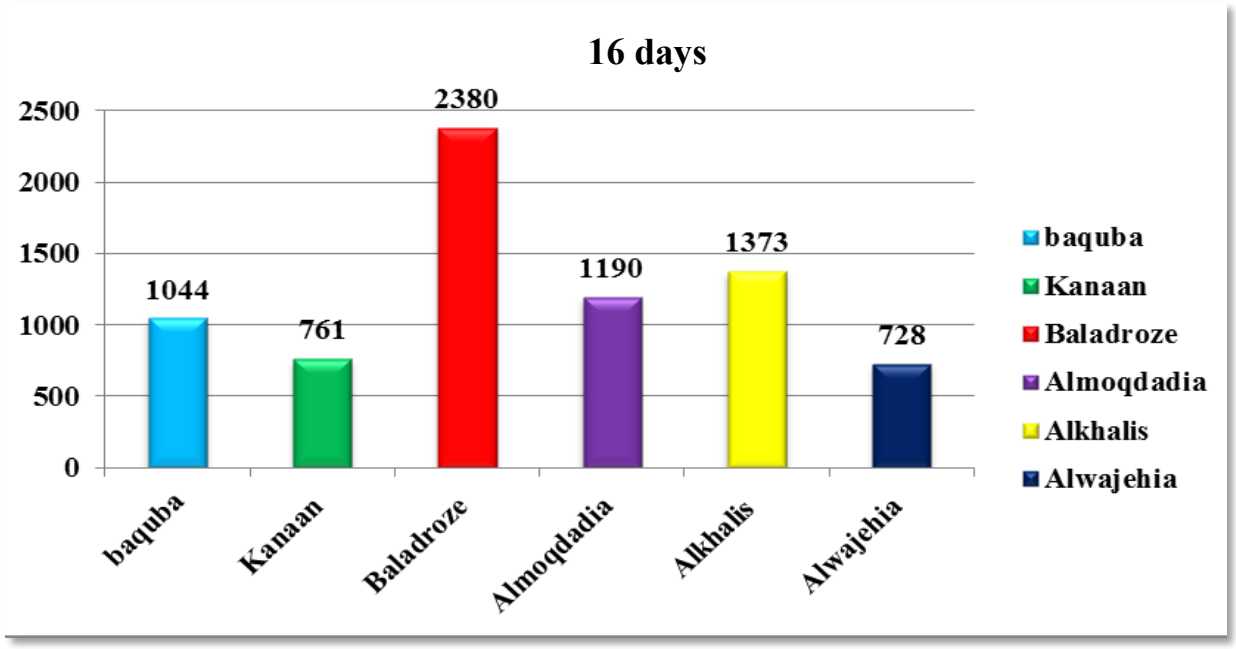


Figure 2. Anti-IBV rate by age groups among infected broiler chickens aged 16 days old in different regions of Diyala Governorate, Iraq

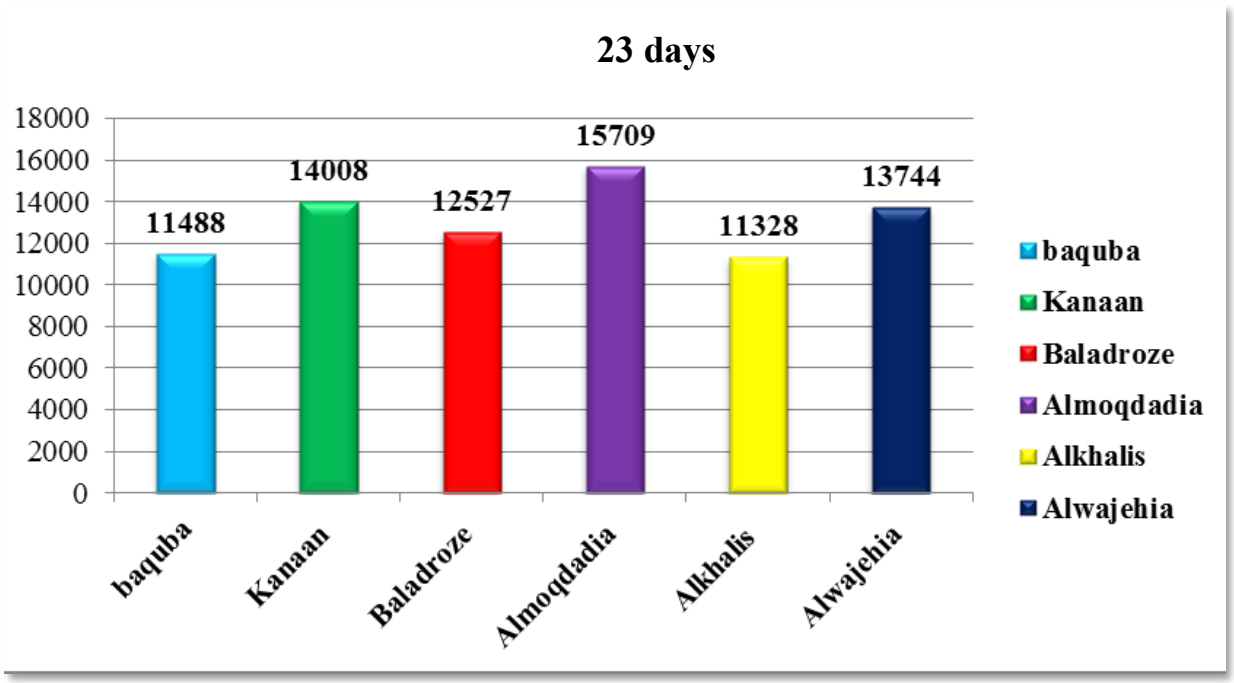


Figure 3. Anti-IBV rate by age group among infected broiler chickens aged 23 days old in different regions of Diyala Governorate, Iraq

Molecular detection of infectious bronchitis virus from tissue samples using RT- PCR

Kidney, liver, trachea, and lung were among the samples collected for molecular analysis. As the samples were subjected to RT-PCR, IBV was found in 25 out of the 30 samples **(**83%**).** A 393bp DNA band was produced in positive samples (Figures 4 and 5).

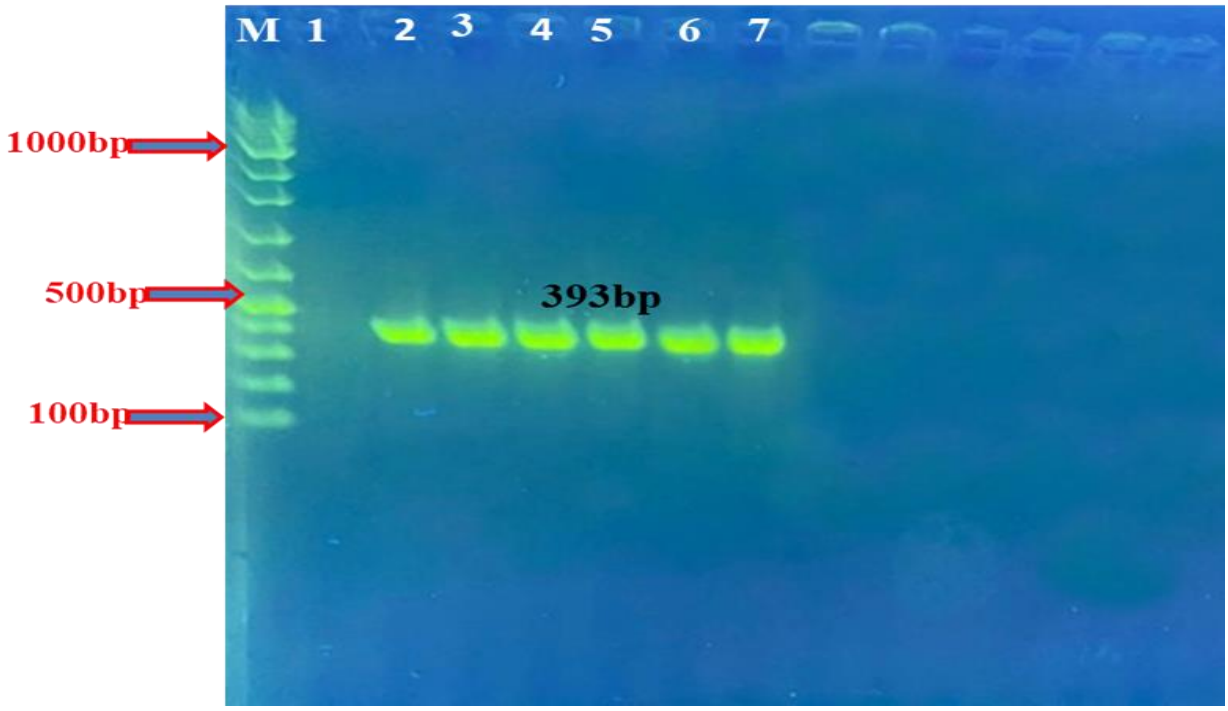


Figure 4. Amplicons of RT-PCR technique for detection infectious bronchitis virus utilizing specific primer pairs. The S1 region of IBV is detected using a pair of primers (SX1 and SX2, SX3 and SX4), which results in a 393-bp PCR product. Electrophoresis was performed on a 1.5% agarose gel. Lane 1 (NTC): Non-template control, Lane 2: Positive control, Lane 3-5: Positive samples, M: 100 bp DNA molecular weight marker.

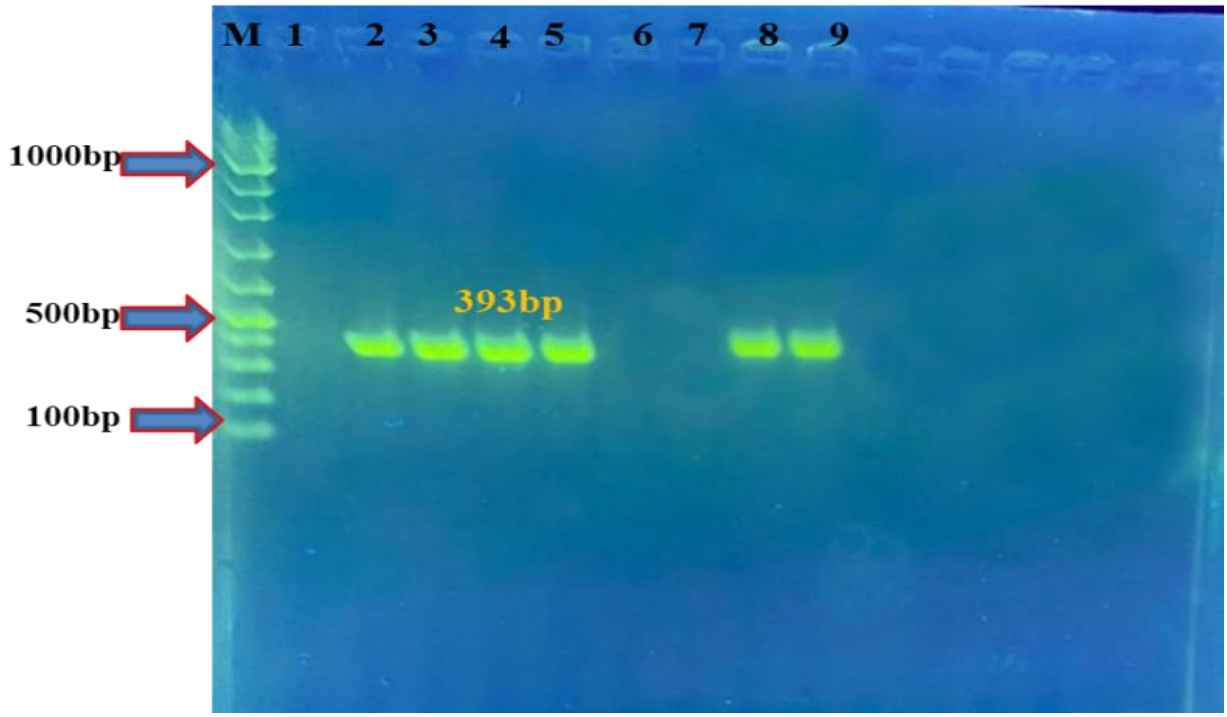


Figure 5. Amplicons of RT-PCR technique for detection infectious bronchitis virus utilizing specific primer pairs. The S1 region of IBV is detected using a pair of primers (SX1 and SX2, SX3, and SX4), which results in a 393-bp PCR product. Electrophoresis was performed on a 1.5 agarose gel. Lane 1 (NTC): Non-template control, Lane 2: Positive control, Lanes 3, 4, 5, 8, 9: Positive samples (IBV), Lane 6, 7: Negative samples. M: 100 bp DNA molecular weight marker.

DISCUSSION

Infectious bronchitis virus is a highly infectious viral disease that affects chicken respiratory and urogenital systems (Cavanagh and Naqi, 2003). The virus has also tissue tropism to kidneys as well as the respiratory and reproductive systems. The IBV is reported all over the world, and it has various varieties with different genomes (Cavanagh, 2007).

Anti-IBV-specific IgG antibodies were identified using commercially available tests (ProFLOK1IBV PLUS ELISA antibody test kit, Synbiotics Zoetis, USA). Based on the OD values, anti-IBV antibody titers were estimated from samples taken from 6 groups (18 chickens in each group) from different farms in Diyala Governorate. The present study showed a high elevation of anti-IBV IgG antibody levels in these flocks (Table 2).

Since the immune system is not well-formed in young chickens, they are susceptible to numerous types of infections (bacterial and viral causes) during the first few days of life (Grindstaff et al., 2003).

In the first eight days following infection, death rates in these groups were low. However, the death rate began to rise after this period. It is possible that once the maternally produced IgG is catabolized, the chicks become more vulnerable (De Wit et al., 2011).

Despite the fact that all flocks were vaccinated (Nobilis® IB4/91vaccine, Netherlands), all six groups of broiler chickens in the current study showed a high level of IgG antibody at 23 days of age. This could be attributed to infection with IBV according to the manufacturer instructions of ELISA kit (Intervet Company, Netherlands) in six different regions of Diyala Governorate. All the investigated flocks were immunized using various vaccination techniques.

Depending on the technique of immunization used by breeders, the vaccine was given through an ocular route, coarse spray, or drinking water but the IBV infection occurred regardless of the vaccination programs. These findings matched those of Bhuiyan et al. (2021), who found that IBV is an acute multiple-system infection in commercial chicken farms because of multiple serotypes that do not cross-protect and so the vaccination cannot cover all environmental serotypes.

The failure of vaccination programs to protect chickens against IBV is due to the inability of chickens to mount a sufficient immune response following inoculation (Bosha and Nongo, 2012). The possibility of long-term immunity, the selection of most virulent serotypes, and the scheduling of applications according to flocks requiring revaccination are all connected with appropriate IBV vaccines. Farm owners blame the ineffectiveness of vaccines to immunize their flocks. More than half of vaccination failures in vaccinated flocks were found to be related to poor vaccine application. Furthermore, the increased risk of vaccine delivery, as well as vaccine maintenance and storage quality, are critical factors for vaccine failure leading to IBV outbreaks in immunized farms (Ganguly et al., 2010; Bosha and Nongo, 2012; Boelm, 2018).

The Nobilis® IB 4 / 91 vaccine (Intervet Company, Boxmeer-Holland) was given to all broiler farms in this investigation although it did not appear to provide complete protection against IBV. This might be due to vaccination failures or the existence of genetic changes that allowed the circulating virus (emerging virus) to evade antibodies generated against the IBV vaccine strain. Furthermore, according to Cavanagh and Naqi (2003), regular vaccination with many IBV strains has been associated with many environmental risk factors that have different influences on a successful vaccination.

The findings of this study also contradicted those of Bhuiyan et al. (2021), who found that IBV exists as multiple serotypes that do not cross-protect, making it extremely difficult to control since there are only a few types of IBV vaccines available for prevention. These findings were also in line with those of Mahmood et al. (2011), and Seger et al. (2016), who expressed that the characterization of IBV has resulted in new issues in terms of epidemiology and control. The IB infections with various varieties in genomes have been discovered around the world in recent decades (Cavanagh, 2007).

Detection of infectious bronchitis virus from tissue samples using RT-PCR

The infectious bronchitis virus's S (spike) gene-encoded protein was used to connect up with the host receptor. The immunization status of the chicken industry may be connected to the biological variety of the viral S gene (Alazawy, 2013; Fraga et al., 2013; Umar et al., 2016). S1 gene sequencing has been an alternative method for distinguishing vaccination from field viruses in recent years (Jones et al., 2011). This (S1) region appears to be preserved in each geographically separated virus, making it useful for viral genotyping. It is the most likely serotype-specific determinant of IBV and contains an antigenic epitope that is serologically significant for IBV serotyping (Mahmood et al., 2011). Due to their sensitivity

and short reaction time, molecular assays are now the most widely used method for IBV detection.

Aside from viral RNA detection, they enable genetic characterization of discovered strains, correct design and evaluation of vaccination programs, and assessment of the existence of specific field strains (Legnardi et al., 2020).

By RT-PCR, 25 out of 30 samples (83%) were positive for IBV. A 393bp DNA band was produced in positive samples. In the current study, there was a high rate of detection (83%), compared to a study by Setiawaty et al. (2019), who found that 26 of 47 (55.3%) samples had a positive result for the S1 gene of IBV using RT-PCR. The detection percentage of IBV by RT-PCR in the current study was close to the findings of Seger et al. (2016) in Iraq, who reported positive samples of IBV by RT-PCR in broiler chickens. The AlNajaf governorate has the highest prevalence of IBV in broiler chickens at 10/20 (50%), and the Al-Muthana governorate has the lowest rate at 4/20 (20%).

CONCLUSION

Infectious bronchitis virus infection was endemic in commercial broiler farms of Diyala Governorate regardless of the control program using commercial vaccines. It seems the maternal antibodies and vaccination did not protect chickens against IBV infection, which might indicate the virus circulation in industrial farms in the study area. Therefore, it is recommended to conduct more molecular studies for local detection of the viruses and the preparation of IBV vaccine from local isolates.

DECLARATIONS

Acknowledgments

This article was not supported by any source of funding. We would like to thank the staff members of the Microbiology Department, College of the Veterinary Medicine/ University of Diyala- Diyala, Iraq) for logistic support.

Authors' contribution

Karrar Awni Jasim, Amer Khazaal Al-Azawy, and Karim Sadun Al-Ajeeli proposed the hypothesis, designed the study, and conducted the serological and molecular works. Karrar Awni Jasim and Talib J.Kadhim collected samples from poultry farms. All authors contributed to manuscript preparation and approved the final manuscript.

Ethical considerations

Ethical issues (including plagiarism, consent to publication, misconduct, data fabrication and/or forgery, double publication and/or submission and replication). Exactly done by all authors.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Alazawy A (2013). Serological study by using the ELISA technique to identification of avian infectious bronchitis disease among some fields of broiler chickens in Diyala Province. Diyala Agriculture Science Journal, 5: 25-37. Available at: https://www.iasj.net/iasj/download/77f385f1e0e83057

Awad F, Baylis M, and Ganapathy K (2014). Detection of variant infectious bronchitis viruses in broiler flocks in Libya. International Journal of Veterinary Science and Medicine, 2(1): 78-82. DOI: https://www.doi.org/10.1016/j.ijvsm.2014.01.001

Bhuiyan M, Alam S, Amin Z, Bakar AMSA, Saallah S, Yusuf NHM, and Siddiquee S (2021). Factor influences for diagnosis and vaccination of avian infectious Bronchitis virus (*Gammacoronavirus*) in chickens. Veterinary Sciences, 8(3): 47. DOI: https://www.doi.org/.3390/vetsci8030047.

Boelm GJ (2018). Influence of maternally derived antibodies on vaccination using a IBV H120 vaccine virus. Journal of Veterinary Medical Research, 5: 1124. Available at: https://www.jscimedcentral.com/VeterinaryMedicine/veterinarymedicine-5-1124.pdf

Boltz DA, Nakai M, and Bahra JM (2004). Avian infectious bronchitis virus: A possible cause of reduced fertility in the rooster. Avian Diseases, 48: 909-915. DOI: https://www.doi.org/10.1637/7192-040808R1

Bosha JA and Nongo NN (2012). Common breaches in poultry vaccine handling and administration in Makurdi metropolis: A recurrent phenomenon in the tropics. Vom Journal of Veterinary Science, 9: 11-16. Available at: https://www.bibliomed.org/?mno=35714

Britton P and Cavanagh D (2007). Avian coronavirus diseases and infectious bronchitis vaccine development. In: V. Thiel (Editor). Coronaviruses: Molecular and Cellular Biology. Caister Academic Press, Norfolk, UK, pp. 161-181.

Cavanagh D and Gelb J (2008). Infectious bronchitis. In: Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE (Editors). Diseases of poultry. Blackwell, Ames, pp. 117-135. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119371199.ch4

Cavanagh D (2007). Coronavirus avian infectious bronchitis virus. Veterinary Research, 38: 281-297. DOI: https://www.doi.org/10.1051/vetres:2006055.

Chhabra R, Chantrey J, and Ganapathy K (2015). Immune responses to virulent and vaccine strains of infectious bronchitis viruses in chickens. Viral Immunology, 28(9): 478-488. DOI: https://www.doi.org/10.1089/vim.2015.0027.

De Wit JJ, Cook JK, and Van der Heijden HM (2011). Infectious bronchitis virus variants: A review of the history, current situation and control measures. Avian Pathology, 40(3): 223-235. DOI: https://www.doi.org/.1080/03079457.2011.566260

Fraga AP, Balestrin E, Ikuta N, Fonseca ASK, Spilki FR, Canal CW, and Lunge VR (2013). Emergence of a new genotype of avian infectious bronchitis virus in Brazil. Avian Diseases, 57(2): 225-232. DOI: https://www.doi.org/10.1637/10346-090412-Reg.1.

Ganguly S, Paul I, and Mukhopadhayay SK (2010). Different types of vaccines and vaccination-the most accepted trend to control and eradicate infections. Indian Pet Journal, 5: 34-37. Available at: https://indianpetjournal.com/home/

Grindstaff JL, Brodie Iii ED, and Ketterson ED (2003). Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1531): 2309-2319. DOI: https://www.doi.org/10.1098/rspb.2003.2485

Jackwood MW and de Wit S (2003). Infectious bronchitis. Diseases of Poultry, 11: 101-119. DOI: https://www.doi.org/10.1002/9781119371199.ch4

Jones RC, Worthington KJ, Capua I, and Naylor CJ (2005). Efficacy of live infectious bronchitis vaccines against a novel European genotype, Italy 02. Veterinary Record-English Edition, 156 (20): 646-647. DOI: https://www.doi.org/10.1136/vr.156.20.646.

Jones RM, Ellis RJ, Cox WJ, Errington J, Fuller C, Irvine RM, and Wakeley PR (2011). Development and validation of RT-PCR tests for the detection and S1 genotyping of infectious bronchitis virus and other closely related *Gammacoronaviruses* within clinical samples. Transboundary and Emerging Diseases, 58(5): 411-420. DOI: https://www.doi.org/10.1111/j.1865-1682.2011.01222.x.

Jordan B (2017). Vaccination against infectious bronchitis virus: A continuous challenge. Veterinary Microbiology, 206: 137-143. DOI: https://www.doi.org/10.1016/j.vetmic.2017.01.002

Kahya S, Coven F, Temelli S, Eyigor A, and Carli KT (2013). Presence of IS/1494/06 genotype-related infectious bronchitis virus in breeder and broiler flocks in Turkey. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 60(1): 27-31. DOI: https://www.doi.org/10.1501/Vetfak_0000002549

O'Connell J (2002). RT-PCR Protocols. Volume 193, Edited by Joe O'Connell, Humana Press, Totowa, New Jersey, pp. 59-240.

Legnardi M, Tucciarone CM, Franzo G, and Cecchinato M (2020). Infectious bronchitis virus evolution, diagnosis and control. Veterinary Sciences, 7(2): 79. DOI: https://www.doi.org/10.3390/vetsci7020079

Liu SW, Zhang QX, Chen JD, Han ZX, Liu X, Feng L, and Tong GZ (2006). Genetic diversity of avian infectious bronchitis coronavirus strains isolated in China between 1995 and 2004. Archives of Virology, 151(6): 1133-1148. DOI: https://www.doi.org/10.1007/s00705-005-0695-6

Liu S, Chen J, Chen J, Kong X, Shao Y, Han Z, Feng L, Cai X, Gu S, and Liu M (2005). Isolation of avian infectious bronchitis coronavirus from domestic peafowl (Pavo cristatus) and teal (Anas). Journal of General Virology, 86: 719-725. DOI: https://www.doi.org/10.1099/vir.0.80546-0

Mahmood ZH, Sleman RR, and Uthman AU (2011). Isolation and molecular characterization of Sul/01/09 avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. Veterinary Microbiology, 150: 21-27. Available at: https://www.doi.org/isc/abstract/20113179932

Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, and Swayne DE (2008). Diseases of poultry, 12th edition. Iowa State University, Press, Ames, Iowa, pp. 117-130. Available at: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781119371199.fmatter1

Schalk AF, and Hawn MC (1931). An apparently new respiratory disease in baby chicks. Journal of the American Veterinary Medical Association, 78: 413-422. Available at: https://eurekamag.com/research/013/304/013304856.php

Seger W, GhalyanchiLangeroudi A, Karimi V, Madadgar O, Marandi MV, and Hashemzadeh M (2016). Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Archives of Virology, 161(5): 1229-1237. DOI: https://www.doi.org/.1007/s00705-016-2790-2

Setiawaty R, Soejoedono RD, and Poetri ON (2019). Genetic characterization of S1 gene of infectious bronchitis virus isolated from commercial poultry flocks in West Java, Indonesia. Veterinary World, 12(2): 231. Available at: http://www.veterinaryworld.org/Vol.12/February-2019/7.html

Steel RG, and Torrie JH (1980). Principles and procedures of statistics: A biometrical approach. Second edition, New York, McGraw-Hill, 2: 137-139.

Umar S, Shah MAA, Munir MT, Ahsan U, and Kaboudi K (2016). Infectious bronchitis virus: Evolution and vaccination. World's Poultry Science Journal, 72(1): 49-60. DOI: https://www.doi.org/10.1017/S0043933915002706

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 107-116, June 25, 2022

Research Paper, PII: S2322455X2200012-12
License: CC BY 4.0

DOI: https://dx.doi.org/10.36380/jwpr.2022.12

Phenotypic Characters and *TYRP1* Polymorphism of F_4 Golden Kamper Hybrid Chickens (*Gallus gallus domesticus* Linnaeus, 1758)

Gilang Ilham Firmansyah, Ayudha Bahana Ilham Perdamaian, and Budi Setiadi Daryono*

Laboratory of Genetics and Breeding, Faculty of Biology, University of Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Sleman Yogyakarta, Indonesia

*Corresponding author's Email: bs_daryono@ugm.ac.id

Received: 07 April 2022
Accepted: 28 May 2022

ABSTRACT

Golden Kamper is a local meat-typed chicken with four generations of Pelung male and Layer female selective breeding. This chicken has various plumage colors and patterns. Therefore, the desired plumage color is red barred plumage (*B1*). In chickens, the missense mutation in the Tyrosinase-related-proteins 1 (*TYRP1*) causes a chocolate color plumage (*choc*) with an epistatic effect on barred plumage. The current study aimed to observe the growth of 16 chickens from hatching until 49 days of age to investigate the phenotypic characteristics, especially plumage color at 49-day-old chickens, then to determine the effect of the *TYRP1* polymorphism on F_4 Golden Kamper phenotypes. The methods used in this study included selective breeding among F_3 Golden Kamper, collection of F_3 Golden Kamper's eggs, then rearing the day-old chickens of F_4 Golden Kamper. Phenotypic data were collected and blood collection was performed for DNA isolation, DNA amplification, and sequencing. Of 16 F_4 Golden Kamper, all chickens had a uniform comb type of single (rprp, 100%). The produced shank colors were white (31.25%), yellow (62.5%), and blackish gray (6.25%). The plumage colors were red barred (12.5%), white barred (12.5%), brown (68.75%), and chocolate (6.25%). The bodyweight of F_4 Golden Kamper at the age of 7 weeks reached 597.3 g. The morphometric results indicated that F_4 Golden Kamper had the same posture and body proportions as Pelung chickens, however, with a higher weight. Fourteen substitutions were found in the *TYRP1* fragment of F_4 Golden Kamper. The single nucleotide polymorphisms (SNP) had no correlation with the chocolate plumage phenotype in F_4 Golden Kamper. The evaluated SNPs in *TYRP1* were not associated with the brown plumage color phenotype.

Keywords: Chicken, Golden Kamper, Phenotype, Polymorphism, *TYRP1*

INTRODUCTION

Pelung is one of the Indonesian local meat-type chickens that originated from Cianjur, West Java province, Indonesia. Pelung chicken has a remarkable superiority in terms of body weight, compared to other local breeds (Henuk and Bakti, 2018). Local chickens have drawbacks in terms of low productivity. Therefore, the farmers prefer to generate commercial broiler and layer chickens for their profits (Ahn et al., 2015; Nurfadillah et al., 2018).

Crossbreeding and selective breeding are genetic approaches that can be used to improve the quality of local chickens. Two individuals who each have superior traits are mated, then the offspring (filial; F_1) are selected based on the desired phenotypic character (Damayanti et al., 2019). Gama Ayam Research Team from the Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University, has conducted selective breeding since 2013 to improve the egg productivity of the local Pelung chickens but retain its the phenotypic characters (Kilatsih et al., 2020; Kurnia et al., 2021).

The hybrid is called the F_1 Kamper chicken which has various phenotypic characteristics. Therefore, selective breeding has been carried out on F_1 Kamper chickens to produce a more uniform F_2 population and continued to derive a uniform F_4 population. The prospective F_1 Kamper and its progenies for full-sib mating are selected based on the character of the red barring trait, brown combined sex-linked barring gene plumage color, and

heavyweight to produce a chicken called F_2 and other types. Crosses between relatives or commonly referred to as inbreeding can lead to a decrease in genetic variation, resulting in uniformity of homozygosity in a population (Antos et al., 2013).

The brown plumage colors in Golden Kamper breeds are common unwanted expressed traits. Brown plumage is derived from genetics and environmental factors interplay. Many scientists had investigated several genes associated with brown plumage (Yu et al., 2017; Makarova et al., 2019; Olori, 2019; Zheng et al., 2020). The Golden Kamper plumage color resembles the chocolate plumage trait in the Orpington breed, a brown layer chicken (Li et al., 2019), and a Rhode Island Red breed. However, the reports of causative mutation of brown plumage (which is also similar to Golden Kamper) at Rhode Island Red are not yet available. Regarding the red barring traits, *TYRP1* is a more precise target, compared to other major brown color genes. Dark brown plumage in Golden Kamper is visually more similar to Chocolate plumage trait Tyrosinase-related-proteins 1 (*TYRP1*) than dark brown (SOX10, Gunnarsson et al., 2011), yellow (SOX10, Zhu et al., 2022), and buttercup (MC1R, Kerje et al., 2003).

Red barred plumage is a black-brown strip caused by dilution of sex-linked barring with brown genes. The barring plumage traits (B0, B1, B2) are caused by a mutation in Cyclin-dependent kinase inhibitor 2A (CDKN2A). The CDKN2A and *TYRP1* are located in Z chromosome (Hellström et al., 2010; Schwochow et al., 2017; Li et al., 2017). Furthermore, Tyrosinase (TYR) which is involved in the same melanin pathway as *TYRP1* has an epistatic effect on barred plumage (Hua et al., 2021).

In chickens, c.640C > A polymorphisms in the exon 3 of *TYRP1* are associated with the appearance of dark brown plumages (chocolate color trait). The current research aimed to investigate the association of *TYRP1* gene polymorphism on F_4 Golden Kamper dark brown plumage color and assess body weight inheritance.

MATERIALS AND METHODS

Ethical consideration

All procedures in this research (rearing, and blood collection) were conducted in accordance with standard chicken care guidelines. No experimental action was conducted in this research.

Chicken breeding and day-old chicken maintenance

The present research was conducted in Center for agrotechnology Innovation (Pusat Inovasi Agroteknologi; PIAT), Kali Tirto, Berbah, Sleman Regency, Yogyakarta, Indonesia. The parental mating was conducted in a cage (8 m^2) and fed with a commercially available pellet as a standard adult feeder (AD-II; Japfa Comfeed) and water *ad libitum*. A total of 16 chickens used in this study were days old chickens (DOCs) of F_4 Golden Kamper produced from female F_3 and male F_3 Golden Kamper mating. All eggs were artificially incubated and the hatched DOCs were transferred into a rearing cage. Adaptation was done for one day and the rearing cages were warmed before DOCs were deployed. Adaptation of DOCs was performed by adding 5mg anti-stress (Vita stress, Medion Farma) and 5 mg multivitamin supplement (vitamins A, B1, B2, B6, B12, C, D3, E, K3 as well as calcium-D-pantothenate, nicotinic acid, natrium butirat) of Vitachick (Medion Farma) in every 7 liters drinking water for a day. The DOCs were reared with lighting and heater using 10 watts light bulb for 24 hours and fed with a crumble standard broiler grower (BR-I; Japfa Comfeed) and water *ad libitum*. As can be seen in Table 1, the quantitative characteristics observed in the current study were body weight which was measured once every week with a digital scale KrisChef EK9350H for 7 weeks and the qualitative characters were measured using a tape measure (Metline) based on Damayanti et al. (2019).

Blood collection and DNA isolation

In the current study, a whole blood sample (1 ml) was drawn from a wing vein using a 3 ml syringe with a 23G needle from all chickens. The collected blood samples were stored in a vacutainer and preserved at -20°C. The DNA was extracted using the Chelex method according to the previous study by Ernanto et al. (2018). Blood was absorbed into Whatman filter paper and then incubated in lysis buffer (200 µL 5% chelex; 18 µL 0.05 M Dithiothreitol (DTT), 2 µL proteinase K [10 mg/mL]) at 100ºC for 8 minutes. The tube was vortexed and incubation was prolonged at 56°C for 2 hours with vortexed and spun down every 15 minutes. Incubation continued at 100°C for 8 minutes then vortexed. Tubes were centrifuged (Gyrozen Mini Centrifuge GZ-1312, South Korea) at 13000 × g for 3 minutes and its supernatant was transferred into clean microtubes. Tris-EDTA (TE) Buffer (1:1) was added to the tube and stored at -20°C.

Table 1. Morphological characters of chickens

Characteristic	Detailed procedure
Chicken height	Measured from the digit/hallux to the tip of the comb
Body height	Measured from the digit/hallux to the end of the distal vertebrae
Beak width	Measured from articular to dexter
Beak length	Measured from the base of the angular process to the end of the mandibular symphysis
Head length	Measured from the supraorbital bone to premaxilla
Head width	Measured from quadratojugal sinister to dexter
Comb height	Measured from the highest tip of the comb to the base of the comb
Comb length	Measured from the back to the front of the comb
Body length	Measured from the tip of the first thoracic vertebra to the base of the pygostyle
Body width	Measured from the base of the femoral bone to dexter
Chest circumference	Measured from the sternal of the keel in a circle
Dorsal length	Measured from the thoracic vertebrae to the caudal vertebrae end
Wingspan	Measured from the base of the humerus to the end of the carpus
Neck length	Measured from the base of the atlas to the tip of the thoracic vertebrae
Tibia length	Measured from the tip of the femur to the base of the tibiotarsus
Femur length	Measured from the end of the patella to the base of the femur
Shank	Measured from the tarsus to the base of the patella

Modified from Damayanti et al. (2019).

Fragment gene of interest amplification and sequencing

The fragment gene of interest was amplified using a gradient thermocycler (BioRad, US) with a specific primer (IDT, Malaysia). A 25 µL cocktail consisted of a 12.5 µL Master mix PCR kit (KAPATaqTM; US), 2.5 µL forward (5'-TCTCATTATTATTCCGTCAGG-3), and reverse (5'-GCAAAGTTCCAGTAGGGTAG-3') primer (Zheng et al., 2020), 2 µL DNA template (± 50 ng/µL), and 8 µL ddH_2O. The amplification protocol was performed as one cycle of pre-denaturation condition at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 30 seconds. Post extension for 5 minutes at 72°C. The PCR product quality was verified by electrophoresis using 2% gel agarose. This step was mandatory to verify the PCR product purity and size before undergoing Sanger sequencing.

The PCR product was sequenced using sanger sequencing (1st BASE, Malaysia) to visualize the single nucleotide polymorphism (SNP). The *TYRP1* (1500 bp) was sequenced with the same primers for PCR. Gene Studio (GeneStudio ver. 2.2.0) and Clustal Omega (2022) were used to observe the presence of SNP.

Data analysis

All data from F_1 to F_4 generation were tabulated and compared with ANOVA and followed by post hoc Tukey HSD using IBM SPSS (version 25) software to assess the significance between generations from hatching to 49 days of age. The observation of plumage color, shank color, and comb shape character were performed at 7 weeks of age. Data were presented in tables and figures. The correlation between SNP and brown plumage color was analyzed using Fisher's Exact Test. P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Pedigree and quantitative phenotype of F_4 Golden Kamper

A cross between F_3 Golden Kamper produced 16 F_4 Golden Kamper DOCs consisting of 7 males and 9 females (Figure 1). The weights of F_4 Golden Kampers were compared with F_1 Kampers (Lesmana, 2016; unpublished data), F_2 Golden Kamper (unpublished data), Pelungs, and layer chickens (Figure 2). As can be seen, F_4 Golden Kampers at the age of 7 weeks had a higher weight (597.3 gr), compared to F_2 Golden Kampers (435.7 gr), Pelungs (472.6 gr), and layers (424.9 gr). These data could indicate that hybridization and selective breeding methods led to positive results in body weight. However, the average weight of F_4 Golden Kampers was still lower than F_1 Kampers (771.3 gr) which was the first filial of a cross between a Pelung rooster and a Layer female. The body weight of F_4 Golden Kamper was lower than F_1 Kamper, which could be influenced by intrinsic and extrinsic factors. Intrinsic factors are factors that influence from within the body, such as genetic factors. However, extrinsic factors are factors that influence from outside the body, such as environmental conditions, exposure to stress, and the amount of consumed nutrients.

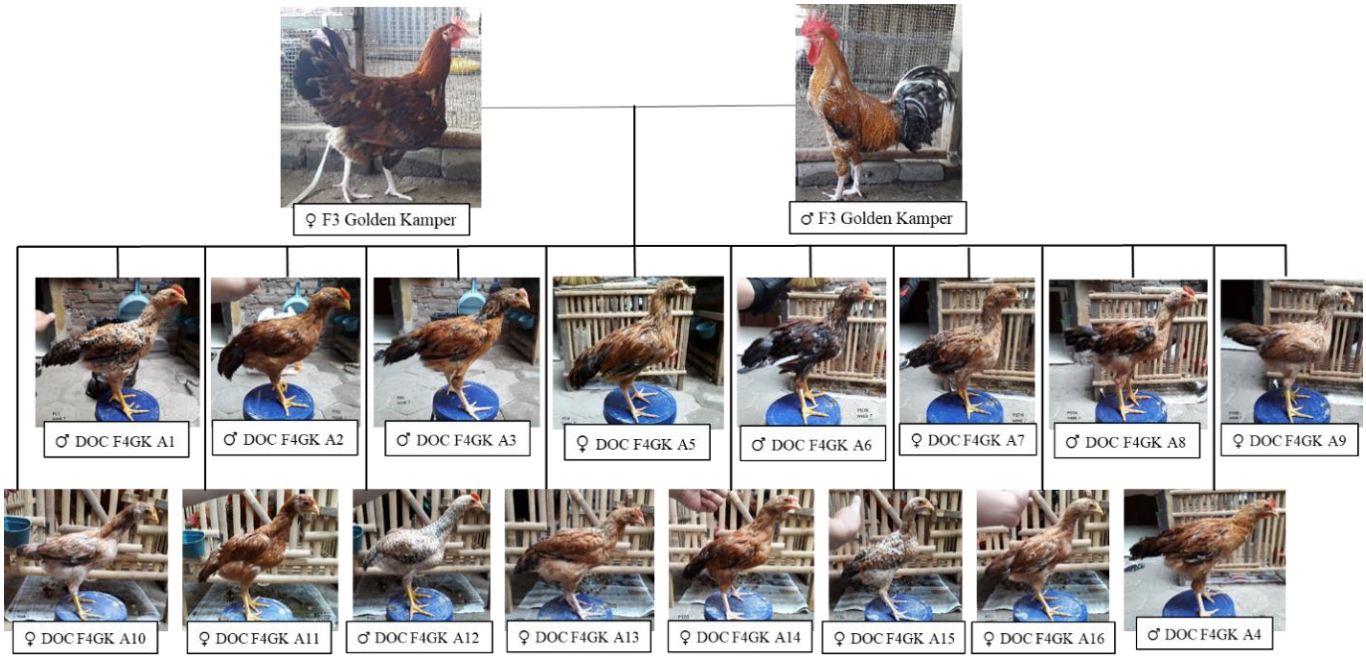


Figure 1. Pedigree and plumage phenotype of 16 evaluated F_4 Golden Kampers

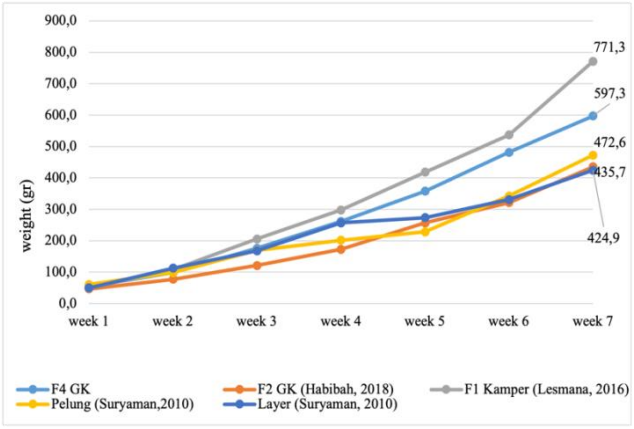


Figure 2. The body weight of chickens from hatching to 49 days of age

The average weight of the five groups of chickens (Pelung, Layer, F_1 Kamper, F_2 Golden Kamper, and F_4 Golden Kamper) was normally distributed. Regarding ANOVA analysis, the average weight of the five groups of chickens was not significantly different ($p > 0.05$). Therefore, although the average body weight of F_4 Golden Kamper chickens was lower than their grandparents (F_1 Kamper), F_4 Golden Kamper chickens remained a prospective local meat-type chicken breed candidate due to the higher body weight, compared to pure Pelung chicken. Body size is a factor that can affect the selling value of local chickens in Indonesia. The proportion of chicken body size can be observed by taking morphometric or zoometric quantitative data of chickens into account (Alsudany et al., 2017). The results of the morphometric measurements of F_4 Golden Kamper can be seen in Table 2.

Table 2. The morphometric characters of F_4 Golden Kamper chicken at 49 days of age

Number	Parameters	Value (cm)
1	Chicken height	32.50
2	Body height	21.94
3	Beak width	1.06
4	Beak length	2.22
5	Head length	1.61
6	Head width	2.03
7	Comb height	2.08
8	Comb length	0.62
9	Body length	8.07
10	Body width	14.31
11	Chest circumference	8.41
12	Dorsal length	5.26
13	Wingspan	20.49
14	Neck length	8.86
15	Tibia length	6.19
16	Femur length	8.97
17	Shank	6.30

F_4 Golden Kamper had similar total height, body height, femur length, and tibia length with Pelung chickens (Mahardhika and Daryono, 2019). Considering total height, F_4 Golden Kamper reached 32.50 cm while

Pelung only 32.13 cm. Regarding body height, F_4 Golden Kamper was higher than Pelung (21.94 versus 20.5 cm), however, F_4 Golden Kamper was shorter than Pelung (6.19 versus 6.79 cm) in terms of femur length. F_4 Golden Kamper had longer tibia than Pelung (8.97 versus 8.90 cm). In case of chest diameter, F_4 Golden Kamper has a larger chest circumference than Pelung (20.49 versus 18.59 cm, Mahardhika and Daryono, 2019). These morphometric data showed that the F_4 Golden Kamper chicken had posture and body proportions that resembled Pelung Chicken, but with a higher weight. This shows that the F_4 Golden Kamper chicken has high potential as an ideal local meat-type chicken because it has the posture and body proportions as Pelung chickens but has a higher body weight.

Qualitative phenotype of F_4 Golden Kamper

In addition to the quantitative characteristics, qualitative characteristics were also investigated in 16 F_4 Golden Kamper chickens. The qualitative characteristics included the shape of the comb, the color of the legs or shank, and the color of body hair (Serpico, 2020). The results obtained from the observation of qualitative characters can be seen in Table 3.

Table 3. Qualitative characters of F_4 Golden Kamper chickens at 49 days of age

Qualitative character	Phenotype (Genotype)	Number	Percentage
Comb Shape	Single (rprp)	16	100
Shank Color	Yellow (*wwZ^{Id}-)*	10	62.5
	White (*W-Z^{Id}-)*	5	31.25
	Blackish-grey (*wwZ^{id}*)	1	6.25
Plumage Color	red-barred *(B1-e^{b})*	2	12.5
	White-barred *(B1-)*	2	12.5
	Brown (*NNe^{b}*-)	11	68.75
	Chocolate- (*NNchoc*)	1	6.25

(A) (B) (C) (D)

Figure 3. Phenotype of F_4 Golden Kamper. A: Red barred, B: White barred, C: Brown, and D: Black-Brown (chocolate).

Based on observations, there are three groups of shank colors on F_4 Golden Kamper, namely white, yellow, and blackish-gray. The group of white shanks consisted of 5 individuals (31.25%), then the yellow shank consisted of 10 individuals (62.5%), and only 1 (6.25%) was categorized as blackish-grey shank chicken. Shank color in chickens is influenced by many different allele genes, including autosomal and sex-linked genes (Jin et al., 2016; Jiguo et al., 2017; Shen et al., 2019). The autosomal dominant *W* gene produces a white color because it inhibits lipochrome, while the recessive *w* allele for *W* produces lipochrome which causes a yellow color in the epidermal layer of the shank. The sex chromosome-linked gene, namely *Id*, acts as a melanin inhibitor, and the recessive allele, namely *id* highlights the black color in the dermis layer of the shank (Daryono and Perdamaian, 2019). Based on observations, it can be concluded that the genotype of a female F_4 Golden Kamper chicken with a blackish-grey shank is *wwZ^{id}*. Then, the genotype of F_4 Golden Kamper with white shank was *W-Z^{Id}Z^{Id}* or *W-Z^{Id}Z^{id}* in males and *W-Z^{Id}* in females. The genotypes of F_4 Golden Kamper with yellow shanks were *wwZ^{Id}Z^{Id}* or *wwZ^{Id}Z^{id}* in males and *wwZ^{Id}* in females. Both parents used in the current study had white shanks, so the genotype of

the female F_3 Golden Kamper shank was WwZ^{Id} and the F_3 Golden Kamper male was $WwZ^{Id}Z^{id}$.

The plumage color of F_4 Golden Kamper is red barring traits ($B1e^b$, 12.5%), White barring traits (B1, 12.5%), Brown (NNE^b, 68.75%), and Black-Brown (chocolate traits; NNchoc, 6.25%). The red barring motif is a plumage pattern of horizontal stripes of two different colors caused by complex temporal and special gene activity (Schwochow et al., 2017). The red barring motif on the F_4 Golden Kamper chicken consists of two or more colors, namely white, black, and brown. This barring motif is inherited from the Pelung *blirik* (white sex-linked barring) which was selected to be used as an ancestor in the first cross with female layer chickens. The *blirik* pattern can mainly be found on the tail, neck plumages, and wing plumages. The color of the golden-brown *blirik* plumage is the color that becomes the target character of the Golden Kamper. The emergence of F_4 Golden Kamper individuals with almost entirely brown body plumage color can be caused by the reappearance of Layer plumage phenotype characteristics. The Lohmann Brown layer chicken elder which was chosen as the ancestor in the previous cross had a brown body color with no *blirik* motif.

Polymorphisms of *TYRP1* in F_4 Golden Kamper

The PCR product of 16 F_4 Golden Kamper and both parental (F_3 Golden Kamper) is illustrated in Figure 4. In both parental and filial, the length of DNA fragment was 1500 bp. This is the length of the nucleotide where the specific primer for *TYRP1* is attached.

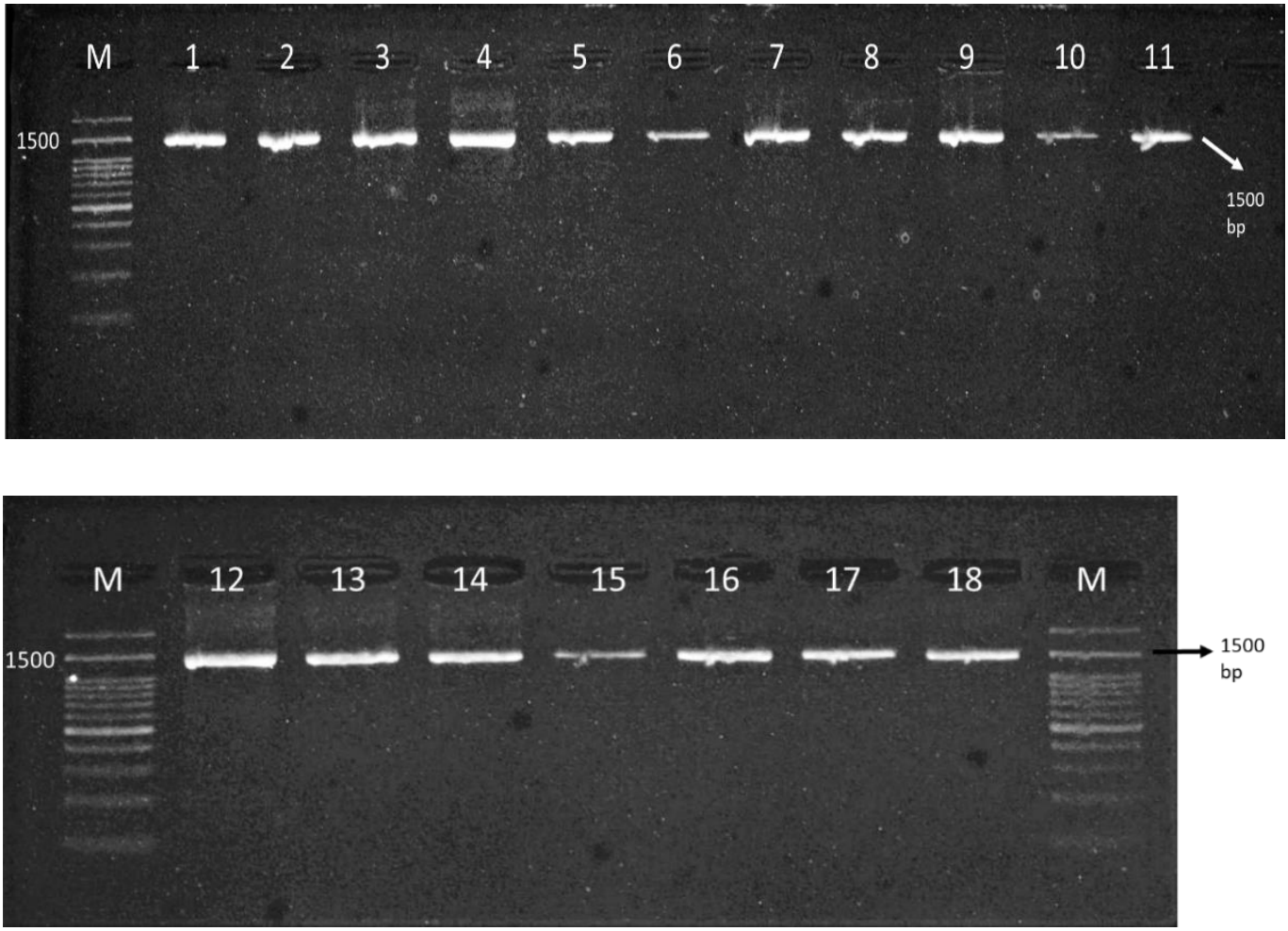


Figure 4. Visualization of 1500 bp PCR product *TYRP1*. M: DNA ladder, 1-16: F_4 Golden Kamper chicken, 17: Male F_3 Golden Kamper, 18: Female F_3 Golden Kamper.

Table 4. The 4-haplotype derived from 14 single nucleotide polymorphisms at *TYRP1*

	Polymorphism of *TYRP1*						
	Reference	**A1**	**A2**	**A6**	**A11**	**P Male**	**P Female**
Single nucleotide polymorphism							
G3536A	G	G	G	G	A	A	A
G3587A	G	G	G	G	A	G	A
C3695T	C	C	C	C	T	C	T
G3738A	G	G	G	G	A	A	A
C3775T	C	C	C	C	T	C	T
T4053G	T	T	T	T	G	G	G
G4186T	G	G	G	G	T	G	T
C4251G	C	C	C	C	G	C	G
A4273G	A	A	A	A	G	A	G
G4460A	G	G	G	G	A	G	A
A4486T	A	A	A	A	T	A	T
C4513T	C	C	C	C	T	T	T
G4773T	G	G	G	G	T	T	T
A4856C	A	A	A	A	C	C	C
Haplotipe	Reference	Reference	Reference	Reference	1	2	1
Plumage Color	-	Red barred	Brown	Chocolate	Brown	Red barred	Chocolate

A: Allele, P: Parental

Based on the results of sequencing and alignment of the *TYRP1* gene, it can be observed that there are 14 mutation points in F_4 Golden Kamper and its parental (F_3 Golden Kamper). The polymorphism points of the *TYRP1* gene are presented in detail and divided into haplotypes (Table 4). Based on Table 4, it can be observed that all polymorphisms that occur in F_4 Golden Kamper and F_3 Golden Kamper brooders are substitution polymorphisms. Substitutions that occur are transversion substitution and transition substitution. All the single nucleotide polymorphisms (SNPs) obtained formed two haplotypes in Golden Kamper and its parental. Chicken codes A1, A2, and A6 have the same DNA sequence as the GeneBank reference (Gene ID: 395913), so it can be assumed that there is no polymorphism in these individuals. The A11 has the same haplotype as the female parent (P Female), while the male parent (P Male) has a different haplotype from the A11 and P Female. Based on the haplotype analysis of the sequencing results of the *TYRP1* gene above, A11 inherits the *Z* chromosome from the female parent because *TYRP1* gene located in *Z* chromosomes (Table 4). The sex of chickens is determined by the *Z* and *W* chromosomes. In contrast to the human sex chromosomes, chickens that have heterogametic chromosomes are female (*ZW*) while male chickens have homogametic sex chromosomes (*ZZ*, Lawal et al., 2020).

The correlation between changes in the nucleotide arrangement due to the presence of polymorphisms with the appearance of the brown plumage color phenotype in F_4 Golden Kamper chickens can be analyzed by Fisher's Exact Test. Correlation test was carried out at each point of the polymorphism of the plumage color of F_4 Golden Kamper. The results of Fisher's exact test are shown in Table 5.

The obtained results indicated that all 14 SNPs in the *TYRP1* were not correlated with the appearance of the brown plumage color phenotype in F_4 Golden Kamper chickens. Therefore, the *TYRP1* gene cannot be used as a molecular marker of the brown plumage color phenotype, an unwanted color that appears in Golden Kamper chicken breeds.

The absence of a correlation between the *TYRP1* gene polymorphism and the brown plumage color phenotype in F_4 Golden Kamper chickens can be caused by multiple factors. In this report, no mutation in the previously reported site (c.640C > A) of *TYRP1* was responsible for the chocolate plumage trait. In chickens, c.640C > A polymorphisms in the exon 3 of *TYRP1* substitute histidine for asparagine amino acid. This mutation occurs in the ZnA region which interacts with zinc metal ions as a cofactor (Solano, 2018) and has a negative effect on the function of the *TYRP1* protein (Li et al., 2019). This mutation is associated with the appearance of dark brown plumages (chocolate color trait) in Orpington chickens.

Chicken plumage color is influenced by a complex variety of genes (Makarova et al., 2019). In addition to the *TYRP1* gene, there are also mutations in other genes that can cause the brown plumage color phenotype in chickens. Schwochow et al. (2021) reported that a 15-bp deletion in the *PMEL17* gene causes a grayish-brown color (dun) in chickens crossed between Red Junglefowl males and White Leghorn females. Meanwhile, based on research from Gunnarsson et al. (2011), an 8.3-kb deletion in the *SOX10* gene causes a dark brown phenotype in hybrid red

jungle fowl chickens. According to a study by Zhang et al. (2015), several genes that control plumage color and skin color in chickens can be specific in certain populations.

Table 5. Correlation test of *TYRP1* polymorphism to brown plumage color phenotype

Polymorphisms	Genotype	Genotype frequency	Brown plumage frequency
G3536A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
G3587A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
C3695T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
G3738A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
C3775T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
T4053G	TT	0.75	0.05
	TG	0	0
	GG	0.25	0.05
G4186T	GG	0.75	0.05
	GT	0	0
	TT	0.25	0.05
C4251G	CC	0.75	0.05
	CG	0	0
	GG	0.25	0.05
A4273G	AA	0.75	0.05
	AG	0	0
	GG	0.25	0.05
G4460A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
A4486T	AA	0.75	0.05
	AT	0	0
	TT	0.25	0.05
C4513T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
G4773T	GG	0.75	0.05
	GT	0	0
	TT	0.25	0.05
A4856C	AA	0.75	0.05
	AC	0	0
	CC	0.25	0.05

CONCLUSION

In conclusion, the obtained results of the current research indicated the benefits of cross breeding and genetics selection for improving the body weight of the local chickens. However, the results revealed that the inheritance fashion of plumage color was complex. It is, therefore, important to conduct further experiments using more target genes associated with eumelanin synthesis .

DECLARATIONS

Acknowledgments

This research was funded by the Ministry of Higher Education Republic of Indonesia (Kemenristekdikti) through the Excellent Applied Research for Higher Education (Penelitian Terapan Unggulan Perguruan Tinggi) Fund 2021 (No.: 7260/UN1. DITLIT/DIT-LIT/PT/2021). Authors also would like to express their gratitude to Gama Ayam Research Team, Mr. Suryadi, and Pusat Inovasi Agroteknologi (PIAT) UGM for their kind assistance during this research work.

Authors' contribution

Gilang Ilham Firmansyah conducted the experiment, writing the original article. Ayudha Bahana Ilham Perdamaian wrote and revised the manuscript. Budi Setiadi Daryono designed the experiment, supervised the study, and revised the manuscript. All authors checked the data and the final draft of the manuscript.

Competing interests

The authors have no competing interests.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical consideration

The authors checked for ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

REFERENCES

Ahn NTL, Kunhareang S, and Duangjinda M (2015). Association of chicken growth hormone and insulin-like growth factor gene polymorphism with growth performance and carcass traits in Thai broiler. Asian-Australasian Journal of Animal Sciences, 28(12): 1686-1695. DOI: https://www.doi.org/10.5713/ajas.15.0028

Alsudany SM, Shahrbabak HM, Ashtiani SRM, and Sadeghi M (2017). Association of chicken MC1R gene polymorphism

with coat colour trait in Iraqi native chicken. Life Science Journal, 14(12): 71-75. DOI: https://www.doi.org/10.7537/marslsj141217.10

Antos P, Andres K, and Kapkowska E (2013). Preliminary studies on genetic diversity of selected polish local chicken varieties. Journal of Central European Agriculture, 14(1): 11-22. DOI: https://www.doi.org/10.5513/JCEA01/14.1.1147

Clustal Omega (2022). Available at: https://www.ebi.ac.uk/Tools/msa/clustalo/

Damayanti PA, Daryono BS, and Mahardhika IWS (2019). Inheritance and comparison of phenotypic characters from Hybrid Chicken GK-Bro (*Gallus gallus* Linnaeus, 1758). Biogenesis, 7(2): 94-99. DOI: https://www.doi.org/10.24252/bio.v7i2.9493

Daryono BS and Perdamaian ABI (2019). Characterization and Genetics diversity of Indonesian Local Chicken. UGM Press, Yogyakarta p. 51. Available at: https://ugmpress.ugm.ac.id/en/product/biologi/karakterisasi-dan-keragaman-genetik-ayam-lokal-indonesia

Ernanto AR, Afifah D, Lesmana I, and Daryono BS (2018). Isolation of DNA from chicken (*Gallus gallus domesticus* Linnaeus, 1758) feather with lysis buffer-phenol chloroform isoamyl alcohol method (PCI) and chelex method, AIP Conference Proceedings 2002, 020002. DOI: https://www.doi.org/10.1063/1.5050098

Gunnarsson U, Kerje S, Bed'hom B, Sahlqvist AS, Ekwall O, Tixier-Boichard M, Kampe O, and Andersson L (2011). The dark brown plumage color in chickens is caused by an 8.3-kb deletion upstream of SOX10. Pigment Cell and Melanoma Research, 24(2): 268-274. DOI: https://www.doi.org/10.1111/j.1755-148X.2011.00825.x

Hellström AR, Sundström E, Gunnarsson U, Bed'Hom B, Tixier-Boichard M, Honaker CF, Sahlqvist AS, Jensen P, Kämpe O, Siegel PB, Kerje S, and Andersson L (2010). Sex-linked barring in chickens is controlled by the CDKN2A/B tumour suppressor locus. Pigment Cell Melanoma Research, 23(4): 521-530. DOI: https://www.doi:10.1111/j.1755-148X.2010.00700.x

Henuk YL and Bakti D (2018). Benefits of promoting native chickens for sustainable rural poultry development in Indonesia. ANR Conference Series, 1(2): 69-76. DOI: https://www.doi.org/10.32734/anr.v1i1.98

Jiguo X, Lin S, Xinfeng G, Nie Q, Luo Q, and Zhang X (2017). Mapping of Id locus for dermal shank melanin in a Chinese indigenous chicken breed. Journal of Genetics, 96(6): 977-983. DOI: https://www.doi.org/10.1007/s12041-017-0862-z

Jin S, Lee JH, Seo DW, Cahyadi M, Choi NR, Heo KN, Jo C, and Park HB (2016). A major locus for quantitatively measuredshank skin color traits in korean native chicken. Asian-Australas Journal Animal Science, 29(11): 1555-1561. DOI: https://www.doi.org/10.5713/ajas.16.0183

Kerje S, Lind J, Schütz K, Jensen P, and Andersson L (2003). Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken. Animal Genetic, 34(4): 241-248. DOI: https://www.doi.org/10.1046/j.1365-2052.2003.00991.x

Kilatsih R, Perdamaian ABI, Trijoko, Purwanto S, and Daryono BS (2020). Effect analysis of prolactin (*PRL*) gene polymorphisms on chicken egg productivity (*Gallus gallus domesticus*) BC1 from crossbreeding between pelung and layer chicken. Iranian Journal of Applied Animal Science, 10(4): 717-726. Avalaible at: https://www.sid.ir/en/journal/ViewPaper.aspx?id=820006

Kurnia RR, Lesmana I, Ernanto AR, Perdamaian ABI, Trijoko, and Daryono BS (2021). The association of follicle stimulating hormone receptor (fshr) gene polymorphism of on egg productivity in hybrid chicken (gallus gallus gallus, linnaeus 1758). Biodiversitas, 22(3): 1221-1226. DOI: https://www.doi.org/10.13057/biodiv/d220318

Lai X, Wichers HJ, Soler-Lopez M, and Dijkstra BW (2018). Structure and function of human Tyrosinase and Tyrosinase-Related Proteins. Chemistry, 24: 47-55. DOI: https://www.doi.org/10.1002/chem.201704410

Lawal RA, Martin SH, Vanmechelen K, Vereijken A, Silva P, Al-Atiyat RM, Aljumaah RS, Mwacharo JM, Wu D, Zhang Y et al. (2020). The wild species genome ancestry of domestic chickens. BMC Biology, 18(13): 13. DOI: https://www.doi.org/10.1186/s12915-020-0738-1

Li J, Bed'hom B, Marthey S, Valade M, Dureux, A, Moroldo M et al (2019). A missense mutation in TYRP1 causes the chocolate plumage color in chicken and alters melanosome structure. Pigment Cell & Melanoma Research, 32: 381-390. DOI: https://www.doi.org/10.1111/pcmr.12753

Makarova AV, Mitrofanova OV, Vakhrameev AB, and Dementeva NV (2019). Molecular-genetic bases of plumage coloring in chicken. Vavilovskii Zhurnal Genetiki i Selektsii, 23: 343-354. Available at: https://www.vavilov.elpub.ru/jour/article/view/2027

Mahardhika IWS and Daryono BS (2019). Phenotypic performance of Kambro crossbreeds of female broiler Cobb 500 and male pelung blirik hitam. Buletin Veteriner Udayana, 11(2): 188-202. DOI: https://www.doi.org/10.24843/bulvet.2019.v11.i02.p12

Nurfadillah S, Rachmina D, and Kusnadi N (2018). Impact of trade liberization on Indonesian broiler competitiveness. Journal of the Indonesian Tropical Animal Agriculture, 43(4): 429-437. DOI: https://www.doi.org/10.14710/jitaa.43.4.429-437

Picardal JP, Afable FA, Lagman A, Campoto EA, Palada E, and Marcos Jr MB (2015). Phenotypic characterization of native chickens (*Gallus gallus domesticus*) in Eastern Samar, Philippines. IAMURE International Journal of Ecology and Conservation, 15(1): 242-265. DOI: https://www.doi.org/10.7718/ijec.v15i1.1005

Schwochow D, Bornelöv S, Jiang T, Li J, Gourichon D, Bed'Hom B, Dorshorst BJ, Chuong CM, Tixier-Boichard M, and Andersson L (2021). The feather pattern autosomal barring in chicken is strongly associated with segregation at the MC1R locus. Pigment Cell and Melanoma Research, 34(6): 1015-1028. DOI: https://www.doi.org/10.1111/pcmr.12975

Schwochow D, Ring H, Sundstrom E, Cao X, Larsson M, and Kerje S (2017). The evolution of sex-linked barring alleles in chickens involves both regulatory and coding changes in CDKN2A. PLoS Genetics, 13(4): e1006665. DOI: https://www.doi.org/10.1371/journal.pgen.1006665

Serpico D (2020). Beyond quantitative and qualitative traits: Three telling cases in the life sciences. Biology and

Philosophy, 35(34): 1-33. DOI: https://www.doi.org/10.1007/s10539-020-09750-6

Shen X, Wang Y, Cui C, Zhao X, Li D, Zhu Q et al. (2019). Detection of Snps in the melanocortin 1-receptor (MC1R) and its association with shank color trait in Hs chicken. Revista Brasileira de Ciencia Avicola, 21(3): 1-9. DOI: https://www.doi.org/10.1590/1806-9061-2018-0845

Solano F (2018). On the metal cofactor in the Tyrosinase family. International Journal of Molecular Sciences, 19(2): 633. DOI: https://www.doi.org/10.3390/ijms19020633

Zhang J, Liu F, Cao J, and Liu X (2015). Skin transcriptome profiles associated with skin color in chickens. PLoS ONE, 10: e0127301. DOI: https://www.doi.org/10.1371/journal.pone.0127301

Zheng X, Zhang B, Zhang Y, Zhong H, Nie R, Li J, and Zhang H (2020). Transcriptome analysis of feather follicles reveals candidate genes and pathways associated with pheomelanin pigmentation in chickens. Science Reproduction, 10: 12088. DOI: https://www.doi.org/10.1038/s41598-020-68931-1

Zhu T, Liu M, Peng S, Zhang X, Chen Y, Lv X, Yang W, Li K, Zhang J, Wang H et al. (2022) A Deletion Upstream of SOX10 Causes Light Yellow Plumage Colour in Chicken. Genes, 13(327): 327. DOI: https://www.doi.org/10.3390/genes13020327

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 117-123, June 25, 2022

Research Paper, PII: S2322455X2200013-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.13

The Effects of Three Commercial Grower Feeds on Performance, Internal Organs, and Carcass traits in Pullet Chickens

Anthony Henry Ekeocha*, Ademiju Adeolu Aganga, Joshua Femi Oluwadele, and Seun Kolawole Ayoola

Department of Animal Production and Health, Federal University, Oye Ekiti, Nigeria

*Corresponding author's Email: anthony.ekeocha@fuoye.edu.ng

Received: 30 March 2022
Accepted: 25 May 2022

ABSTRACT

Poultry farming is categorized as a developing business venture in most countries, especially Nigeria. This is followed by poultry feed production units ranging from smaller compartments to commercial poultry feed producers. This research study was carried out to examine the physical, and biochemical parameters of feed, growth performance, carcass traits, and visceral organs of pullets fed selected commercial grower feeds and formulated diet. A total number of 1200 *Isa Brown* pullets aged 10 weeks were divided into 4 groups with 5 replicates for each group randomly. This research experiment was completed within 8 weeks. All poultry feeds were filled inside standard polyethylene woven bags in the absence of insects/mold. All poultry feeds, including Top Feed, Chikun Feed, and formulated diet were grouped into mash form except one of the commercial feeds Vital Feed in the pelleted form which is the treatment of the research. There were significant differences in final body weight, weight gain, feed consumed, and feed conversion ratio among the experimental treatments. The least weight was recorded among hens fed Vital feeds with the highest feed intake, which might be due to high fiber content in the feed. The dietary treatment significantly affects the live weight, dressed weight, neck, breast muscle, liver, kidney, gizzard, and abdominal fat of pullet fed different commercial feed and formulated diets. The findings of the current study indicated that a self-formulated diet at the grower stage could replace the commercial poultry feeds used in the study.

Keywords: Body weight, Carcass traits, Grower feed, Pullets, Visceral organs

INTRODUCTION

To control the health and well-being of chickens, proper feeding is essential. In most nations, particularly Nigeria, this is followed by poultry feed production units ranging from small-scale production of feeds to commercial poultry feed producers (Akinola and Ekine, 2018). Ethical feed production practices are established features of small animal industries in developed and developing countries; however, it is quite different in developing countries and executed in different ways. For instance, in Nigeria, there is no definite system for checkmating the quality and quantity of poultry feeds sold to farmers. Poor quality feeds result in a high death rate and low production. These resulted in low returns on capital investment (Griffioen et al., 2020). Many feed producers in Nigeria reduce the nutritional qualities required for poultry by including low-quality feed substances during feed formulations. However, knowing the nutritional requirements has a great value for the digestibility, biochemical composition, and the presence/absence of prominent anti-nutritional factors (Dobermann et al., 2017). Moreover, the essential nutrients in poultry feeds could not remain constant as a result of some factors, such as time, storage period of feed ingredients, and methods of poultry feed processing (Noblet et al., 2021). Feed producers do not usually consider these factors before feed production.

Income obtained for poultry production is greatly dependent on feed utilization, which accounts for 70-80% of the total production cost (Thirumalaisamy et al., 2019). Hence, it is very important to ensure safe feed quality with appropriate nutritional values suitable for effective production (Kalam et al., 2021). The present study aimed to examine the physical, and biochemical parameters of feeds and the performance of selected commercial grower feeds frequently used by farmers.

To cite this paper: Ekeocha AH, Aganga AA, Oluwadele Jf, and Ayoola SK (2022). The Effects of Three Commercial Grower Feeds on Performance, Internal Organs, and Carcass traits in Pullet Chickens. *J. World Poult. Res.*, 12 (2): 117-123. DOI: https://dx.doi.org/10.36380/jwpr.2022.13

MATERIALS AND METHODS

Collection and preparation of test ingredients

The experiment was conducted at the Teaching and Research Farm, Federal University, Oye Ekiti, Nigeria. It has an average annual temperature ranging from 21°C to 28°C with high humidity of over 75%. A general survey was conducted for the poultry feed producers. After the survey, lists of 5 commercial feed brands were chosen. The three most popular brands were purposively chosen from this study and feed superiority was established for brands having close to five brands accepted by farmers within the city for standard utilization and palatability. The selected poultry feed brands were coded with two lettered words Top Feed (TF), Vital Feed (VF), and Chikun Feed (CF). The formulated diet was coded as FF. The batches of sampled poultry feeds did not stay more than one week during storage to reduce feed quality deterioration. Selected commercial poultry feeds were bought from different outlets. Feed samples were taken and analyzed appropriately. For the specific brand of commercial grower feeds, 25 g of feed were taken from each bag purchased at different times during the current study. The feed samples were mixed before chemical analysis. This was carried out for each brand of commercial poultry feeds and formulated diet was produced separately. However, every two weeks of development during the study period, 25 g of formulated feed was obtained for sample analysis and appropriately mixed before chemical analysis for adequate evaluation.

Management of experimental animals

A total of 1200 *Isa Brown* pullets aged 10 weeks were used in this study. Young chickens of the same live body weights were randomly assigned to four dietary treatments. Each group used a brand of commercial grower feed and a formulated diet and was replicated five times. The chickens were housed in battery cages of 300 pullets per treatment and 60 chickens per replicate. The experimental design was a completely randomized design (CRD). Routine and standard management practices were carried out (Kalam et al., 2021). Daily feed requirement was weighed and fed to the chickens as per *Isa brown* and water were supplied *ad libitum*. This study lasted for 8 weeks from July to August 2020.

Experimental diet

There were four dietary experimental groups, namely T_1 (Top Feed), T_2 (Chikun Feed), and T_3 (Vital Feed Diets). T_1, T_2, and T_3 were commercial feeds bought from different commercial producers while diet T4 is a formulated diet (NRC, 1994) serving as a control dietary treatment as shown in Table 1.

Physical examination and proximate analysis of feed

Each feed brand purchased for poultry feed was weighed and recorded. Feed content was also calculated. The nutrient contents displayed on each bag label were recorded to ensure a proper understanding of nutritional content. The categories of feed were either pellet, mash, or crumble and were recorded accordingly. Furthermore, feed ingredient was weighed and examined for the presence of insects/mold, caking and even odors of the feed materials before feed production.

Data collection

Data were collected for feed intake (FI), feed conversion ratio, and body weight gain. Pelleted feed was weighed and supplied to pullets at the commencement of the week. The feed leftover was also weighed at the end of each week to determine FI for the period of the experiment as FI = Feed supply minus feed leftover (FS-FL). Furthermore, the birds were weighed on day 1 for initial body weight, followed by weekly weighing throughout the experimental period.

Table 1. Physical characteristics of the commercial layer feeds used in diet of *Isa brwon* chickens

Brand of feed	Declared wt. (kg)	Actual weight (kg)	Feed texture	Foreign bodies	Mould	Flavor	Cost per kg ($)
TF	25	24.6	Mash	NP	NP	Fresh	0.28
CF	25	24.9	Mash	NP	NP	Fresh	0.27
VF	25	24.8	Crumble	NP	NP	Fresh	0.27
FF	25	25	Mash	NP	NP	Fresh	0.25

NP: Not present, N.B: All feeds were packaged in standard polyethylene woven bags (pwb), wt: Weight. TF: Top feed, CF: Chicken feed, VF: Vital feed, FF: Formulated feed.

Carcass evaluation and organs measurement

After the 8 weeks of the feeding trial, five birds per treatment were randomly selected for analysis of carcass traits and organ parameters (Ekeocha and Afolabi, 2012). Feeds were withdrawn overnight and the chickens were slaughtered using human slaughtered techniques through mild electrical stunning to induce unconsciousness before slaughter, de-feathered, processed, and then eviscerated (Webster et al., 1996). Weights of the carcass, cut-off parts (head, neck, wing, breast, back, thigh, drumstick, and leg), and internal organs (heart, crop, gizzard, spleen, small intestine, large intestine, caeca, abdominal fat, and cloaca) were recorded. Caeca weight was recorded using a sensitive scale and tap rule (Sano et al., 2021). The dressing percentage was calculated. It is determined by dividing the carcass weight by the live weight, then multiplying by 100.

Metabolic trial

A number of 50 birds per treatment were randomly selected for the study. The birds were tagged, weighed, and housed separately in metabolic battery cages. Adequate feeding troughs and drinkers, including the facility for collecting fecal matter, were available. The birds were acclimatized for seven days in the cage and weighed feed was given to the birds for 14 days and their faces were collected each day for 14 days early in the morning. Fecal samples were sundried, weighed, and kept in a polythene bag inside a refrigerator for further analysis.

Statistical analysis

The Methodology Statistical analysis of discrimination issues was made using the Statistical Package for the Social Sciences (SPSS), version 18. The research data obtained from this experimental study were analyzed using a one-way Analysis of Variance (ANOVA). The significant differences among means were tested at a significant level of 0.05 using Tukey's test.

RESULTS AND DISCUSSION

Biochemical evaluation of selected grower feed

Table 2 present the proximate composition concerning the brands of commercial grower feeds. The proximate composition was compared to the displayed feed quantity on the bag labels. No feed producer indicated the moisture content of poultry feeds. However, on laboratory analysis, the feed moisture contents ranged from 4.5 to 6% (4.5% in VF, 5.7% in CF, 6% in TF, and 6% in FF). These values were better than that of poultry feeds stored without deteriorating within a short period of time (3 months).

Table 2. Comparison analyzed and nutrient compositions of different commercial grower feed fed to pullet chickens of *Isa brwon* breed

Parameters \ Feed	TF	CF	VF	FF
Declared moisture (%)	ND	ND	ND	ND
Analysed moisture (%)	6.00^{a}	5.70^{b}	4.50^{c}	6.00^{a}
Declared crude protein (%)	15.00^{a}	15.00^{a}	13.00^{b}	ND
Analysed crude protein (%)	15.57^{d}	16.70^{b}	16.10^{c}	17.50^{a}
Declared fat (%)	5.00^{b}	4.00^{c}	8.00^{a}	ND
Analysed fat (%)	7.10^{a}	7.30^{a}	6.70^{b}	7.10^{a}
Declared fibre (%)	7.00^{b}	6.00^{c}	15.00^{a}	ND
Analysed fibre (%)	5.80^{b}	5.90^{b}	6.20^{a}	5.70^{b}
Declared metabolizable energy (k cal)	2450.00^{b}	2600.00^{a}	2600.00^{a}	ND
Analysed metabolizable energy (k cal)	2300.00^{d}	2400.00^{b}	2350.00^{d}	2500.00^{a}
Declared calcium (%)	1.00^{a}	1.10^{a}	0.90^{a}	0.10^{a}
Analyzed calcium (%)	0.45^{b}	0.53^{b}	0.56^{b}	1.85^{a}

Means on the same row with different superscripts are significantly different ($p < 0.05$). T1, T2, and T3 were commercial feeds bought from different commercial producers while diet T4 is a formulated diet serving as a control dietary treatment. TF: Total feed, VF: Vital feed, CF: Chikun feed, FF: Formulated feed, ND: Not defined

This result was in contrast with the results obtained by (Akinola and Ekine, 2018) who had moisture contents ranging from 10% to 13.35%, which was above the optimum recommended range (10-12%). The high

moisture percentage supported the growth/development of fungi in the feed store for a long period (Sharma, 2019). The result of crude protein content analyzed in hosen grower's feeds was slightly higher than the protein content values displayed on all brands of poultry feeds. Akinola and Ekine (2018) also reported a slight increase in the crude protein value analyzed in broiler feed than the values displayed on brands of poultry feeds. The percentages of the crude fat content of the selected commercial grower feeds were higher than the values displayed on bag labels. Although this serves as a source of energy, excessive fat content in the feed has a limiting effect on poultry birds, for example, prolapse which causes fat deposition in the abdominal region. Furthermore, excessive fat content in the feed also augments oxidative rancidity and possible offensive odor. This led to feeding deterioration within a short period of time.

Laboratory analysis of crude fiber percentage and metabolizable energy for the selected commercial poultry feeds were less than the values displayed on bag labels. The reduction of crude fiber and metabolizable energy values for the selected commercial grower feeds could be ascribed to the high cost of feed ingredients. Hence, poultry feed producers set their goals to maximize their profit through which they may produce low-quality feed.

Performance evaluation

Table 3 presents the performance of pullet hens fed with commercial grower feeds and formulated diet for 8 weeks. The final body weight/weight gain of hens was higher in formulated grower feed (FF) than the bodyweight noted in commercial grower feed (VF, $p < 0.05$). Sharma (2019) reported that during the process of pullets from 7 to 16 weeks of age, the pullets fed with formulated diets added more weight ($p < 0.05$) than pullets fed with the commercial grower diet. This performance could be attributed to the quality of the feed ingredients used in diet formulation. Energy and protein concentrations in the diet pose an important function in livestock productivity and are critical in evaluating poultry performance (Alagawany et al., 2020). It was reported that the genetic ability of chickens can only be realized in case of adequate nutrient intake under some variable environmental conditions (Madhuri et al., 2020). This might require updates to the nutrient recommendations for poultry. Poultry diets must be formulated to give nutrient requirements for chickens if optimum growth is to be achieved (Oluwadele et al., 2020).

There was a significant difference in feed intake ($p < 0.05$) with hens fed with commercial feed (VF). Furthermore, the highest feed conversion ratio was noticed in hens fed with commercial feed (VF), while the least feed conversion ratio was noted in hens fed FF ($p < 0.05$). Sanusi et al. (2015) reported significant differences in growth performance, average daily feed consumed, daily weight gained, and FCR of the chicken fed with a self-formulated diet and four different commercial feed. Compared to high performance in hens fed with VF commercial feed can be linked to the pelleted form of the feed which enhances feed intake. Pelleting is known to improve feed intake in broilers and pullets regardless of the grain source (Mudhunguyo and Masama, 2015). These improvements could be associated with a high density, improved digestibility of starch emanating from chemical changes during the process of pelleting, increased nutrient intake, variations in physical characteristics, reduction in feed wastage, and low energy spent for consumption (Gopi et al., 2019).

The dietary treatment significantly ($p < 0.05$) affected the live weight, dressed weight, neck, breast muscle and thigh of the pullet fed different commercial grower rations and formulated diet. However, no significant effects ($p > 0.05$) were recorded on the dressing factor and organs including the head, wings, back, drumstick, and feet.

Tables 4 and 5 showed carcass traits and visceral organs of pullet hens fed with the chosen commercial grower feeds and formulated diet for eight weeks. This result supported the study conducted by Sanusi et al. (2015), who reported a significant difference in live weight, plucked weight, dressing percentage, carcass weight, and the weight of the pancreas. Similar research observations were also carried out by Sanusi et al. (2015) also reported significant ($p < 0.05$) effects of diet on the gizzard, spleen, and adipose tissue found in the abdomen. The highest gizzard weight was observed in hens fed with formulated diet (FF, 55.20 g) as compared to other dietary treatments, which ranged from 46.8 to 52.4 g. These findings could be linked to high fiber contents in diets of hens fed with VF and FF. These findings were similar to the published reports by Varastegani and Dahlan (2014). They all concluded that feeding high fiber diets can increase the length and weight of intestines, and other visceral organs. The growth of the poultry digestive system, with respect to the gizzard, is also influenced by feed particle size. This development is also obvious in chickens reared to their 7 days of age (Williams et al., 1996). It was reported that greater gizzard shape

development and lower pH in 7-day old chicks fed with medium or coarse particle feed size diets compared with chicks fed with fine particle diets which indicates a steady growth as the chicks are fed (Lee, 2021). The gizzards exert a mechanical pressure which may surpass 585 kg/cm^2 (Rubio, 2018). This may lead to poor gizzard development and enlarged proventriculus when broilers or pullets consume finely grounded, administered diets (Taylor and Jones, 2004). Hence, in these conditions, the gizzard plays the role of a transit organ and not a grinding organ (Zhao et al., 2021). It was observed that hens on diet VF had the lowest carcass quality. This could be attributed to the feeding form and also the low nutrient quality displayed on the feed bag. This could not meet up the nutrient requirement of the birds for optimum performance. Furthermore, this act of including low protein and energy source in the feed is a result of feeding (pelleted) which tend to increase the cost of production, and feed miller has to reduce the quality and quantity of the feed ingredients used in manufacturing the feed in order to minimize the cost of production and maximize profit at the detriment of the chicken performance fed with the balanced diet. Similar findings were reported by Hussein et al. (2001), indicating that highest level of protein which is treatment T2 (Chikun feed). Cost evaluation for a self-formulated diet was cheaper with the least cost of $ 0.24/ kg feed. The present finding was similar to the findings of Apantaku et al. (2006). The highest feed cost of $ 0.38$0.29 per kg was recorded on the VF diet. Sanusi et al. (2015) also reported that a self-formulated diet was easy and cheap to produce and it had the lowest cost of feed. Table 5 presents the visceral features of pullet fed with different commercial feed and formulated diets. Dietary treatment had a significant effect on the weight of liver, kidney, gizzard, and abdominal fat ($p < 0.05$), while the dietary treatment had no significant effect on spleen, heart, proventriculus, lungs, small intestine, large intestine, empty crop, caeca and duodenal loop of the chickens fed with different experimental diets.

Table 3. The effect of commercial grower feeds on the performance of *Isa brwon* pullet chickens

Parameters \ Feed brand	TF	CF	VF	FF
Initial body weight (g/hen)	550	540	540	540
Final body weight (g/hen)	1290^{a}	1250^{ab}	1180^{b}	1340^{a}
Total body weight (g/hen)	4281.2^{b}	4252.08^{b}	4494^{a}	4361.84^{ab}
Weight gain (g/hen)	327.6^{b}	319.76^{b}	283.92^{c}	393.68^{a}
Feed Conversion Ratio	1.22^{b}	1.44^{ab}	1.69^{a}	1.2^{b}

Means on the same row with different superscripts differ significantly ($p < 0.05$). $^{a, b, c}$ means different superscripts in the same column differ significantly ($p < 0.05$). TF: Top feed, CF: Chicken feed, VF: Vital feed, FF: Formulated feed.

Table 4. The effects of dietary treatment with different commercial grower feeds on live weight, dressing percentage, and carcass parameters of *Isa brwon* pullet chickens

Parameters	T_1	T_2	T_3	T_4
Live wt (g)	1360^{ab}	1396^{ab}	1318^{b}	1570.00^{a}
Dressed wt (g)	929.80^{ab}	970.80^{ab}	912.40^{b}	1076.60^{a}
Dressing (%)	68.33	69.57	69.27	68.63
Head (g)	45.60	45.80	50.00	54.60
Neck (g)	71.60^{b}	77.20^{ab}	77.80^{ab}	88.20^{a}
Wings (g)	113.60	133.00	111.20	131.60
Breast (g)	211.40^{ab}	218.00^{ab}	189.80^{b}	257.80^{a}
Back (g)	219.80	233.20	222.00	266.00
Thigh (g)	140.80^{ab}	141.80^{ab}	129.00^{b}	153.60^{a}
Drumstick (g)	121.20	131.80	123.00	139.80
Feet (g)	51.40	52.60	52.40	58.80

Means on the same row with different superscripts different significantly ($p < 0.05$). $^{a\,b\,c}$ means different superscripts in the same column different significantly ($p < 0.05$). Means on the same row with different superscripts are significantly different ($p < 0.05$). T1, T2, and T3 were commercial feeds bought from different commercial producers while diet T4 is a formulated diet serving as control dietary treatment. wt: Weight.

Table 5. The effects of different selected commercial grower feeds on internal organs' weights of *Isa brwon* pullet chickens

Parameters	T_1	T_2	T_3	T_4
Liver (g)	20.20^{ab}	18.20^{b}	19.80^{ab}	23.20^{a}
Kidney (g)	3.80^{ab}	5.20^{a}	5.20^{a}	3.40^{b}
Spleen (g)	2.80	2.20	2.20	2.80
Heart (g)	6.00	7.40	6.20	7.80
Proventriculus (g)	7.00	7.40	7.60	7.80
Gizzard (g)	46.80^{c}	50.40^{bc}	52.40^{ab}	55.20^{a}
Lungs (g)	8.20	7.00	8.20	9.00
Small intestine (mm)	116.80	107.80	105.60	107.60
Large Intestine (mm)	19.90	22.40	21.80	25.60
Crop (g)	7.20	9.40	8.60	7.20
Caeca (mm)	16.10	15.70	14.40	15.20
Duodenum loop (mm)	15.60	15.00	14.60	14.40
Abdominal fat (g)	29.60^{ab}	24.80^{ab}	30.40^{a}	7.80^{b}

Means on the same row with different superscripts are significantly different ($p < 0.05$), T1, T2, and T3 were commercial feeds bought from different commercial producers while diet T4 is a formulated diet serving as control dietary treatment, ^{a,b,c} means different superscripts in the same column differ significantly ($p < 0.05$).

CONCLUSION

The study indicated a self-formulated diet at the grower stage can replace the commercial poultry feeds used in the study. The farmers should attest that using a self-formulated diet at the grower stage would be cheaper and could also enhance the optimum performance and profitability. The replacement of a self-formulated diet with commercial feeds in the study did not cause any negative effect on growth performance, carcass yield, and internal organs.

DECLARATIONS

Acknowledgments

Special thanks go to Tertiary Education Trust Fund (TetFund) Nigeria for funding this research and Mrs. Gode Dakuna for technical assistance in the field.

Authors' contribution

No fund was assigned to the Authors. Seun Ayoola collected the samples and carried out the fieldwork and wrote the first draft. Anthony Ekeocha, Ademiju Adeolu Aganga, and Oluwadele Joshua Femi supervised the overall research, and statistical analysis and revised the draft and final script approved by the authors.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors which command respect in Nigeria by the ethical committed monitory team.

REFERENCES

Akinola LF and Ekine OA (2018). Evaluation of commercial layer feeds and their impact on performance and egg quality. Nigerian Journal of Animal Science, 20(2): 222-231. DOI: https://www.doi.org/10.4314/tjas.v20i2

Alagawany M, Elnesr SS, Farag MR, Tiwari R, Yatoo MI, Karthik K, Michalak I, and Dhama K (2020). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health - a comprehensive review. The Veterinary Quarterly, 41(1): 1-29. DOI: https://www.doi.org/10.1080/01652176.2020.1857887

Apantaku SO (2006). Analysis of participation of farmers in participatory poultry production research in Lagos State, Nigeria. Livestock Research for Rural Development, 18: 1-12. DOI: https://www.doi.org/10.4236/JSS.2006.934134

Dobermann D, Swift JA, and Field LM (2017). Opportunities and hurdles of edible insects for food and feed. Nutrition Bulletin, 42(4): 293-308. DOI: https://www.doi.org/10.1111/nbu.122

Ekeocha AH and Afolabi KD (2012). Carcass characteristics of broilers fed mexican sunflower (*Tithonia diversifolia*) leaf meal-based diets. Report and Opinion, 4(9): 51-55. DOI: http://www.dx.doi.org/10.7537/marsroj040912.09

González-Alvarado JM, Jiménez-Moreno E, Lázaro R, and Mateos GG (2007). Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive

performance and digestive traits of broilers. Poultry Science, 86(8): 1705-1715. DOI: https://www.doi.org/10.1093/ps/86.8.170

Gopi M, Purushothaman MR, Dhinesh Kumar R, Prabakar G, and Chandrasekaran D (2019). Ubiquinol supplementation on energy metabolism and oxidative stress in broiler chicken. Indian Journal of Animal Research, 4(54): 445-450. DOI: https://www.doi.org/10.18805/ijar.B-3523

Griffioen H, Booij T, and Doerr C (2020). Quality evaluation of cyber threat intelligence feeds. In Conti, M. et al. (eds) Applied Cryptography and Network Security: 18th International Conference, ACNS 2020, Rome, Italy, October 19-22, Springer, Cham, 277-296. DOI: https://www.doi.org/10.1007/978-3-030-57878-7 14.

Hussein AS, Cantor AH, Pescatore AJ, Gates RS, Burnham D, Ford MJ, and Paton ND (2001). Effect of low protein diets with amino acid supplementation on broiler growth. Journal of Applied Poultry Research, 10(4): 354-362. DOI: https://doi.org/10.1093/japr/10.4.354

Kalam MA, Alim MA, Shano S, Nayem MR, Badsha MR, Mamun MA, Hoque A, Tanzin AZ, Khan SA, Islam A et al. (2021). Knowledge, attitude, and practices on antimicrobial use and antimicrobial resistance among poultry drug and feed sellers in Bangladesh. Veterinary Sciences, 8(6): 111. DOI: https://www.doi.org/10.3390/vetsci8060111

Lee SA, Febery E, Mottram T, and Bedford MR (2021). Growth performance, real-time gizzard pH, and calcium solubility in the gut of broiler chickens is dependent on the interaction between dietary calcium concentration and limestone particle size. British Poultry Science, 62(6): 827-834. DOI: https://www.doi.org/10.1080/00071668.2021.1929840

Madhuri G, Swathi G, Radhakrishna P, and Nagalakshmi D (2020). Effect of replacement of antibiotics with probiotics on performance, carcass characteristics and nutrient retention in broilers fed with meat cum bone meal. Indian Journal of Animal Research, 12(54): 1565-1571. Available at: https://arccjournals.com/journal/indian-journal-of-animal-research/B-3915

Mudhunguyo A and Masama E (2015). Comparison of broiler chicken performance on different phase feeding programs. International Journal of Innovative Research and Development, 4(6): 404-408. Available at: http://www.internationaljournalcorner.com/index.php/ijird_ojs/issue/current

National Research Council (NRC) (1994). Nutrient requirements of poultry: Ninth revised edition. Washington, DC: The National Academies Press. DOI: https://www.doi.org/10.17226/2114.

Noblet J, Wu SB, and Choct M (2021). Methodologies for energy evaluation of pig and poultry feeds: A review. Animal Nutrition, 8: 185-203. DOI: https://www.doi.org/10.1016/j.aninu.2021.06.015

Popoola IO, Popoola OR, Adeyemi A, Ojeniyi O, Olaleru I, Oluwadele F, and Akinwumi E (2020). Overall performance, carcass yield, meat safety potentials and economic value of heat-stressed broilers fed diets with balanced electrolytes. Food and Nutrition Sciences, 11: 615-628. DOI: https://www.doi.org/10.4236/fns.2020.117044

Rubio AA (2018). Effects of Corn particle size and feed form on growth performance and carcass characteristics of broilers. Available at: https://www.etd.auburn.edu//handle/10415/6279

Sano Y, Kanbe H and Kihara M, (2021). Morphological changes of pyloric caeca and their relevancy to motility in laboratory-reared Kurosoi rockfish (Sebastes schlegelii Hilgendorf), using an in vitro assay method. Aquaculture, 539.736604. DOI: https://doi.org/10.1016/j.aquaculture.2021.736604

Sanusi M, Rabi A, Doma UD, and Haruna J (2015). Comparative effects of self-formulated and four commercial diets on the growth performance, carcass and haematological parameters of broiler finisher in the tropics. Sokoto Journal of Veterinary Science, 13(2): 14-19. DOI: https://www.doi.org/10.4314/sokjvs.v13i2.3

Sharma M (2019). Performance of different commercial layer feeds on egg. Journal of Krishi Vigyan, 7: 184-189. DOI: https://www.doi.org/10.5958/2349-4433.2019.00031.X.

Taylor RD and Jones GPD (2004). The incorporation of whole grain into pelleted broiler chicken diet. Gastrointestinal and digesta characteristics. British Poultry Science, 45: 237-246. DOI: https://www.doi.org/10.1080/00071660410001715849

Thirumalaisamy G, Muralidharan J, Senthilkumar S, Hema Sayee R, and Priyadharsini M (2019). Cost-effective feeding of poultry. International Journal of Environmental Science and Technology, 5: 3997-4005. Available at: https://www.ijset.net/journal/1410.pdf

Varastegani A and Dahlan I (2014). Influence of dietary fiber levels on feed utilization and growth performance in poultry. Journal of Animal Production Advances, 4(6): 422-429. Available at: http://psasir.upm.edu.my/id/eprint/34183/

Webster AB, Fletcher DL, and Savage SI (1996). Humane on-farm killing of spent hens. Journal of Applied Poultry Research, 5(2): 191-200. DOI: https://www.doi.org/10.1093/japr/5.2.191

Williams RB, Bushell AC, Répérant JM, Doy TG, Morgan JH, Shirley MW, Yvoré P, Carr MM, and Frémont F (1996). A survey of *Eimeria* species in commercially-reared chickens in France during1994. Avian Pathology, 25(1): 113-30. DOI: https://www.doi.org/10.1080/03079459608419125

Zhao T, Wang S, Zou X, Zhang H, Yan Y, Huo H, Wang Q, and Su C (2021). Synthesis and up-conversion luminescence properties of Ho3+-Yb3+ co-doped glass ceramics containing Sr3Gd (PO4)3. Optical Materials, 121: 111547. DOI: https://www.doi.org/10.1016/j.optmat.2021.111547

JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 124-132, June 25, 2022

Research Paper, PII: S2322455X2200014-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.14

Genetic Characterization of Co-circulated Classic and Very Virulent Infectious Bursal Disease Viruses in Commercial Broiler Flocks of Egypt

Ali Zanaty, Zeinab Mossad*, Mahmoud Said, Mohamed Samy, Fatma Amer, Neveen Rabie, and Mohamed Ahmed Soliman

Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute, Agriculture Research Center (ARC), Giza. 12618, Egypt

*Corresponding author's E-mail: drzienabmosaadvet@gmail.com

Received: 07 April 2022
Accepted: 02 June 2022

ABSTRACT

In recent years, the reintroduction of the infectious bursal disease virus (IBDV), particularly its severe strains, has imposed considerable cost on the Egyptian poultry industry. The goal of the current study was to investigate the molecular features of IBDV in Egypt from June 2019 to April 2021. A total of 30 field samples (bursa of Fabricius) were collected from broiler farms in which the chickens were vaccinated (Transmune 2512 s/c) at hatching. A highly variable region encompassing VP2 gene was targeted for IBDV screening utilizing reverse transcription-polymerase chain reaction (RT-PCR). Of 30 tested samples, 16 were positive by PCR. To isolate the virus, the bursal suspension was injected into 10-11 day embryonated chicken eggs via the chorioallantoic membrane. Five current positive isolates from 2021 were chosen for nucleotide and amino acid (aa) sequence analysis. Phylogenetically, three of the strains under study belonged to the very virulent (vvIBDV) strains, with 97-98% resemblance to Giza 2008 belonging to the (Genogroup 3) IBDV strain. The remaining two strains were identified as a vaccination strain (genotype 1) that matched the winter field 2512 vaccine strain by a similarity percentage of 98. Mutations in the antigenic locations of (P) domain loops were discovered when the sequencing samples were compared to the existing IBD vaccines. The circulating strains were found to be very similar to vvIBDV serotype 1 genotype 3 strains with mutations in the P domain loop providing a potential reason for the circulation of vvIBDV viruses in Egyptian broiler farms despite the vaccination program.

Keywords: Bursa, Classic infectious bursal disease strain, Virulent infectious bursal disease, Virus protein 2 Gene

INTRODUCTION

Infectious bursal disease is an immune-suppression infectious fowl disease resulting in a wide range of opportunistic illnesses with a reduced response to immunization (Eterradossi and Saif, 2013; Sajid et al., 2021). The disease is caused by the infectious bursal disease virus (IBDV) and is distinguished by bursal atrophy. The IBDV is belong to Avibirnaviridae group of *Birnaviridae* family which is a double-stranded RNA virus of two segments (A and B). Serotype 1 is the most recognized cause of avian diseases (Sajid et al., 2021). Segment (A) cleaves into two major proteins of pVP2 and VP3. The pVP2 functions as a major capsid protein IBDV encoded by the VP2 gene. The outbreaks of IBD are still reported worldwide as a significant immunosuppressive disease of fowls. A wide variety of serotype-I have been classified as virulent and very virulent groups (van den Berg et al., 2004; Jackwood and Sommer-Wagner, 2011). Genetic re-assortment and mutations are the factors that change the antigenicity and increase viral virulence. Meanwhile, the IBDV properties, such as heat and chemical resistance, have arisen difficulty with the disease control (Jackwood et al., 2008).

The economic losses in the poultry industry caused by IBD lead to the mortality and morbidity of the flock and a dramatic decline in their productivity. Suppression of humoral immunity was the result of IBDV infection due to B-lymphocytes depletion (Vukea et al., 2014). Thus, the IBD-infected flocks would be more vulnerable to opportunistic pathogens. The annual economic loss in the

meat chicken industry is measured at 3-4 million kilograms (Shabbir et al., 2016; Ray et al., 2021). The agent of IBD is present in clinical, subclinical, and carrier forms of infected flocks, and vaccination and biosecurity are the only ways to prevent IBD (Hussain et al., 2004; Khan et al., 2017). The antigenic drift has created classical and variant antigenic groups. The antigenic groups of IBDV are determined by the hypervariable region (HVR) ofVP2 (hvVP2) into four loop structures, namely PBC, PDE, P_{FG}, and P_{HI}. Antigenic drift induces the IBD vaccine ineffective due to minute mutations in the HVR of the VP2 gene. The re-assortment of two segments of A and B advocated the extension of the virus (Metwally et al., 2009; Mawgod et al., 2014; Fan et al., 2020).

The IBDV subtypes are differentiated using RT-PCR. The DNA sequencing of the HVR VP2 can differentiate classic, variant, and vvIBDV strains as they have characteristic nucleotide and amino acid substitution (Islam et al., 2012; Fan et al., 2020; Aliyu et al., 2021) .The amino acids within the range of 206-350 are chosen for sequence of HVR-VP2 and to characterize IBDV strains. Alterations in antigenicity are analyzed for the examination of differences that occur spontaneously or by attenuation in various strains, resulting in virulence changes (Jackwood et al., 2008; Aliyu et al., 2021).

Since its introduction in Egypt in 1989, very virulent IBDV has been recorded in vaccinated Egyptian flocks (El-Batrawi, 1990). In the last decade, Egypt has been overwhelmed with multiple IBD outbreaks despite the use of various vaccines (Mohamed et al., 2014; Shehata et al., 2017; Eladl et al., 2020). Therefore, the increase in the new variant IBDV strains has turned into a threat in recent years, which requires an update about the genetic properties of the circulating field isolates of IBDV. With this in mind, the current study was conducted from 2019 to 2021 for molecular identification and viral isolation of IBDV infection from vaccinated broilers flocks. In addition, selected isolates were selected for genome sequencing of the hypervariable area in the VP2gene, which could provide more up-to-date genetic information and possible mutation analyses useful for updating control tactics.

MATERIALS AND METHODS

Field samples

Bursal samples (10 bursae of Fabricius/farm) were collected from 30 broiler chicken farms located indifferent provinces of Egypt. These farms indicated a mortality rate of 20-30% and a high morbidity rate of 60%. The chickens were vaccinated at one day old by subcutaneous injection of an IBDV intermediate strain vaccine (Transmune2512ceva®/France) which was a mixed live winterfield 2512 IBDV with hyperimmune specific anti-IBDV immunoglobulins. Bursal samples were stored at -20℃ until further analysis.

Infectious bursal disease virus screening in field sample

Sample preparation

The kanamycin was added to phosphate-buffered saline to prepare 10% tissue suspensions of bursal homogenate. The tissue suspension was frozen three times and then centrifuged at8000 rpm for 5 minutes. In the next step, cell debris was removed and the supernatant was filtered using a 0.45µm filter (Sartorius, Germany) and then stored in a -80°Cfreezer until use (Yovel et al., 2008).

Molecular screening by RT-PCR

RNAs were directly extracted from stored suspension using QiaAmp® Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. AgPath-ID™ One-Step RT-PCR Reagents Kit (Applied Biosystems, USA) on the extracted RNA was performed to process the reverse transcriptase-polymerase chain reaction (RT-PCR). Forward and reverse primers were used F/AUS GU: 5-TCACCG TCCTCAGCTTACCCACATC-3 and R/AUS GL: 5GGATTTGGGATCAGCTCGAAGTTGC-3 to amplify a 620 bp included in HVR of the VP2 gene (Metwally et al., 2009).

The cycling conditions for amplification of PCR product included one cycle for reverse transcription at 48°C for 30 minutes followed by 95°C for 10 minutes, then 40 cycles at 95°C for 30 seconds, 59°C for one minute, and 72°C for 90 seconds with a final extension cycle at 72°C for 10 minutes. Analysis was performed by gel electrophoresis 1.5% against 100 bp Plus DNA Ladder GeneRuler™ (Fermentas).

Virus isolation

The stored suspensions of positive samples were inoculated to the chorioallantoic membrane of the embryonated eggs on days 10-11. Then, they were incubated at 37°C with candling daily. The allantoic fluids were collected at 4-5 days post-inoculation after cutting the egg shell and shell membranes at the blunt end of the eggs by pushing the suction bulb until the whole volume of allantoic liquid was acquired. Afterward, PCR testing was applied to confirm isolation (Dufour-Zavala, 2008).

Sequence and phylogenetic analysis of VP2 gene hypervariable region

Of field isolates, five were selected for gene sequencing in 2021 (Table 1). These isolates underwent molecular identification by RT-PCR as mentioned above. PCR products were visualized by Gel electrophoresis using excision of expected band 620bp, and purification with the QIA quick Gel Extraction Kit (QIAGEN, USA) according to the protocol given by the manufacturer. Sequencing of purified PCR products was performed using Big Dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA, USA) followed by purification using a spin column Centrisep® kit (Applied Biosystems, USA). Sequence chromatograms were retrieved from 3500 genetic analyzer (Applied Bio-systems, Life technologies, Thermofisher, USA). To determine and compare the obtained sequence identity to those published IBDV strains in GenBank, a BLAST® search (Basic Local Alignment Search Tool) was performed(Abdel-Alim et al., 2003).Nucleotide sequence data of HVR of VP2 gene were analyzed by Bio Edit software. A Meg Align module of Laser gene DNA Star was used to determine sequence identities among analyzed strains. Neighbor-joining phylogenetic tree using Egyptian viruses and other international reference and vaccine strains was constructed using maximum composite likelihood model with 1000 bootstrap inMEGA6 (Tamura et al., 2013). The five sequences were then deposited in the NCBI-database and the accession numbers were listed (Table 1).

Table 1. Epidemiological data and accession number of VP2 of infectious bursal disease virus isolates strains in the current study

Samples Name	Province	Collection Year	Host	Age (days)	Vaccination age (Vaccine)	Gene Bank Accession No.	Phenotype
EGY-VVIBDV-GIZA83-2021-VP2	Giza	2021	Broiler	25	Transmune 2512	OK092295	very virulent strain
EGY-CK-IBDV-GIZA84-2021-VP2	Giza	2021	Broiler breeder	26	Transmune 2512	OK092296	Classical
EGY-CK-IBDV-MONOFIA85-2021-VP2	Monofia	2021	Broiler	33	Transmune 2512	OK092297	very virulent strain
EGY-CK-VVIBDV-DAKH87-2021-VP2	Dakahlia	2021	Broiler breeder	28	Transmune 2512	OK092298	very virulent strain
EGY-CK-IBDV-DAKH88-2021-VP2	Dakahlia	2021	Broiler	35	Transmune 2512	OK092299	Classical

RESULTS

Clinical and postmortem findings

The infected chickens aged 3 to 5 weeks were characterized by ruffled feathers, severe depression, trembling, white watery diarrhea, severe prostration, vent picking, anorexia, and dehydration. On postmortem examination, the afflicted chickens exhibited swollen, edematous, yellowish, and occasionally hemorrhagic cloacal bursae. Carcasses were severely dehydrated with congestion and hemorrhage of the pectoral and thigh muscles was observed.

Infectious bursal disease virus screening

Using RT-PCR, it was found that 16 of 30 field samples (53%) were positive for IBDV. The RT-PCR confirmed the presence of IBDV in the inoculated embryos. Embryos that died three days post-inoculation (13-14 days of age) showed severe congestion, dwarfing muscle hemorrhages, and enlarged congested internal organs.

RT-PCR and sequencing of Infectious bursal disease virus hyper variable region

For the five IBDV isolates, the 620bp nucleotide and 206 amino acid sequence flank the HVR were described. However, for the sequencing studies, only the 525bp nucleotide and 175aa sequence of the VP2 HVR of the IBDV isolates were analyzed (Figure 1). The original sequence was investigated to exclude ambiguous nucleotide sequences that were common at the beginning of a sequencing process. The nucleotide locations 625-1044 and amino acid positions 211-350 of the HVR described the isolates were covered by all the identified and sequenced strains of IBDV.

Hyper variable region phylogenetic analyses

Phylogenetic analyses created for HVR sequences revealed that the sequences established two large clusters, which corresponded to the two suggested genogroups (1

and 3). Three of the five sequences (OK092295, OK092297, and OK092298) grouped with the other Egyptian very virulent IBDV (vvIBDV-G3), which is often detected worldwide (Figure 1). The remaining two strains under study (OK092296 and OK092299) established a cluster with the classic cvIBDV-G1 together with Thia14, blue strain, and Winterfield2512 sequences (Figure 1).

Hyper variable region mutational analysis

When the VP2 HVR of five IBDV strains was sequenced, it was determined that three of them were related to Egyptian (vvIBDV-G3) and shared high amino acid similarity (94-98%) with each other and to Giza 2008 strains (acc.no:EU584433.2) which represent Egyptian vvIBDV-G3. However, when compared to traditional genogroup 1 (G1) vaccine strains, 93% identity percent was discovered (Table 2). The nucleotide identity percent of the strains (OK092296 and OK092299, Table 1) was 97.3 to IBDVs from G1 (2512-W). The amino acid similarity of these two isolates was 96% (Table 2). These isolates exhibited the amino acid sequence found in classical strains and 2512-W (Figure 2).

Except for the substitution L217S in the strain (OK092299) under research, all of the amino acid residues of the HVR (212D, 213D, 217L (loop PBC), 242V, 256V (Loop PDE), (270T and 299N) were identical to those of 2512-W strains. As a result, the isolates had a higher level of amino acid similarity than the 2512-W strain (Figure 2).

Meanwhile, the three isolates (OK092295, OK092297, and OK092298) had amino acids characteristics identified in highly virulent IBDV strains (Figure 2). Furthermore, the isolate exhibited some particular amino acid changes, including Y220 F, D213N, G254D, S315T, S317R (Loop PHI), and A321E (Loop PHI), which have been described in some Egyptian-vvIBDV strains (egy-Giza-2008 acc.no EU584433.2). In all very virulent strains, the presence of the Ssp I restriction site correlates to a substitution at residue 294 (leucine to Iisoleucine). As revealed in the amino acid alignment report, the Ssp I restriction site on VP2 has shown to be indicative of vvIBDV strains (Figure 2). The VP2 HVR of all research isolates retains the virulence signature of serine-rich heptapeptide sequences (SWSASGS), which is proximal to (B, P_{HI}) the major hydrophilic peak. All of the strains obtained in the current study had glutamine at position 253 rather than histidine.

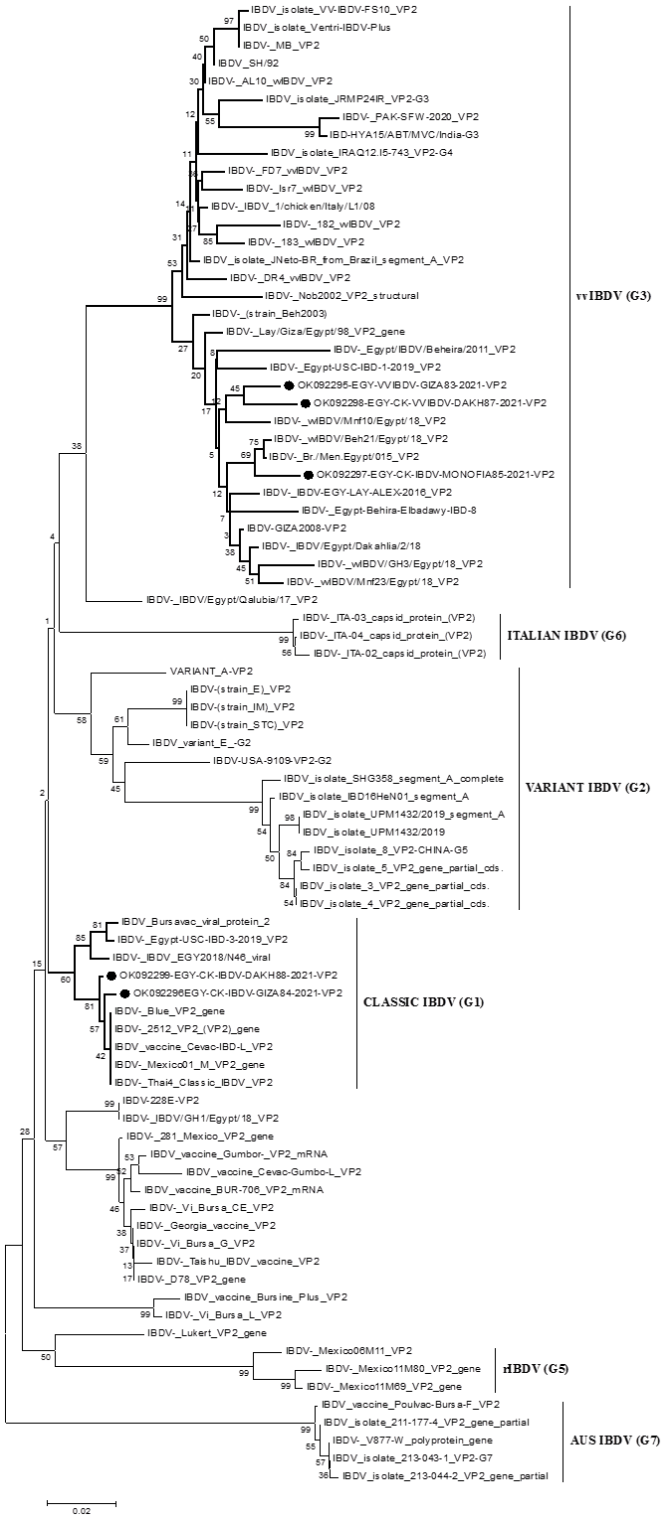


Figure 1. Neighbor-joining phylogenetic tree with 1000 bootstrap repeats tests phylogeny method and Tamura 3-parameter model of the nucleotide sequences of 620bp IBDV-VP2 gene hypervariable region. The five IBDV isolates in the study marked as a black circle.

Table 2. The Nucleotide and Amino acids similarity between infections bursal disease virus isolates under study and other Egyptian and representative reference strains obtained from NCBI

	IBDV VP2 HVR Nucleotide identity (%)																		
Isolate name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1. IBDV-GIZA2008-VP2		96%	93%	93%	86%	94%	75%	73%	98%	98%	92%	93%	94%	95%	96%	95%	91%	92%	1
2. IBDV- Egypt/IBDV/Beheira/2011 VP2	97%		92%	91%	84%	91%	73%	71%	96%	96%	89%	91%	92%	93%	93%	93%	90%	90%	2
3. IBDV- Blue VP2 gene,	96%	94%		100%	89%	98%	80%	76%	94%	93%	87%	97%	96%	93%	92%	94%	97%	97%	3
4. IBDV- 2512 VP2 (VP2) gene,	95%	93%	99%		89%	98%	80%	76%	93%	93%	87%	96%	95%	93%	92%	94%	97%	97%	4
5. IBDV-228E-VP2	87%	86%	89%	88%		89%	84%	82%	86%	85%	93%	90%	88%	88%	87%	89%	90%	90%	5
6. IBDV Bursavac viral protein 2	94%	92%	97%	97%	88%		79%	76%	94%	93%	88%	97%	96%	93%	92%	94%	96%	96%	6
7. IBDV vaccine Cevac-IBD-L VP2	76%	75%	80%	80%	83%	77%		95%	75%	74%	81%	77%	76%	77%	76%	77%	81%	81%	7
8. IBDV vaccine Bursine Plus VP2	73%	72%	74%	74%	80%	73%	92%		73%	73%	79%	76%	76%	75%	75%	76%	77%	78%	8
9. IBDV- Lay/Giza/Egypt/98 VP2 gene,	99%	97%	96%	95%	87%	94%	76%	73%		98%	91%	93%	94%	95%	95%	95%	92%	92%	9
10. IBDV- Egypt-USC-IBD-1-2019 VP2	99%	97%	96%	95%	87%	94%	76%	73%	99%		91%	92%	93%	95%	95%	95%	91%	91%	10
11. IBDV- (strain Beh2003)	92%	89%	88%	87%	93%	86%	81%	78%	91%	91%		87%	87%	92%	91%	92%	88%	89%	11
12. IBDV- D78 VP2 gene	93%	91%	96%	96%	89%	96%	77%	74%	93%	93%	86%		95%	92%	91%	93%	94%	94%	12
13. IBDV variant E -G2	93%	91%	93%	93%	86%	93%	73%	72%	93%	93%	85%	93%		93%	92%	93%	93%	94%	13
14. OK092295-EGY-VVIBDV-GIZA83-2021-VP2	97%	93%	93%	93%	88%	92%	78%	74%	96%	96%	92%	91%	91%		96%	97%	95%	95%	14
15. OK092297-EGY-CK-IBDV-MONOFIA85-2021-VP2	98%	95%	94%	93%	89%	92%	77%	74%	97%	97%	94%	91%	91%	98%		96%	93%	93%	15
16. OK092298-EGY-CK-VVIBDV-DAKH87-2021-VP2	98%	94%	94%	94%	89%	93%	78%	75%	97%	97%	93%	92%	91%	98%	99%		94%	95%	16
17. OK092296EGY-CK-IBDV-GIZA84-2021-VP2	92%	91%	97%	96%	89%	94%	81%	75%	92%	92%	88%	93%	90%	94%	94%	94%		99%	17
18. OK092299-EGY-CK-IBDV-DAKH88-2021-VP2	93%	93%	97%	96%	90%	95%	81%	76%	93%	93%	89%	94%	91%	96%	95%	96%	98%		18
IBDV VP2 HVR Amino acids identity (%)																			

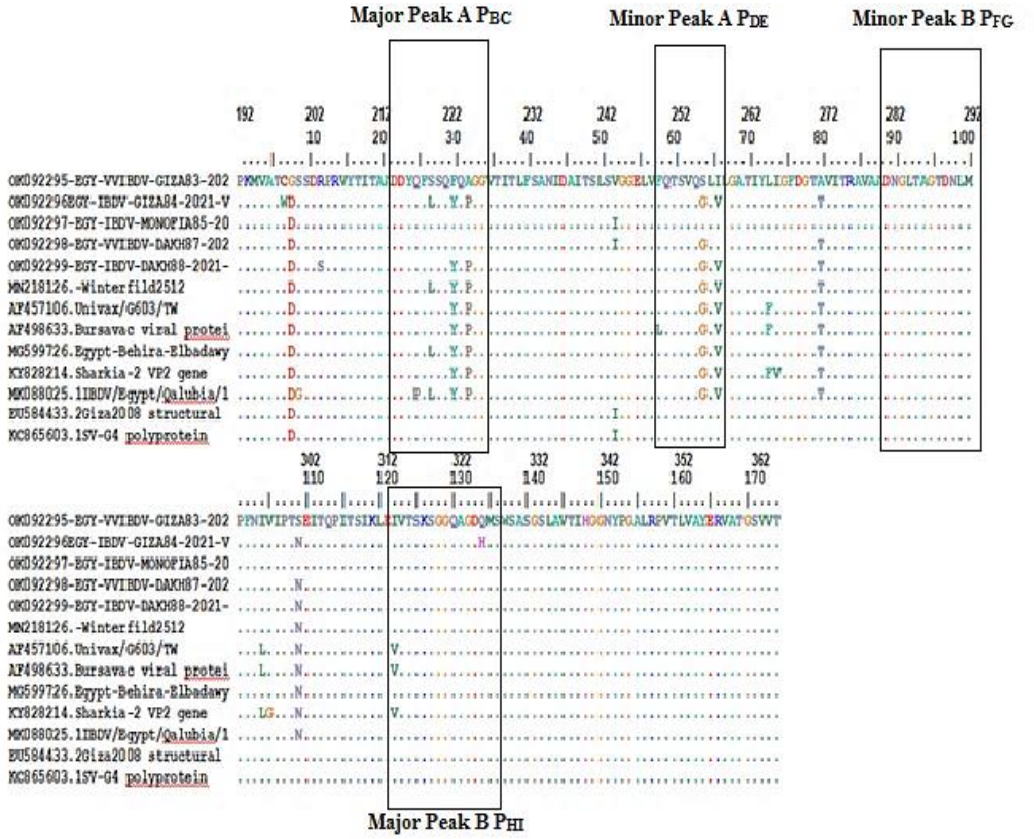


Figure 2. Amino acids alignment report of partial sequence of VP2 gene of IBDV and other Egyptian and representative vaccinal strains

DISCUSSION

The rapid mutation of IBDV's double-stranded RNA, compared to DNA viruses causes alterations in the hvVP2 protein, which is thought to be the most important factor for antigenicity (Domingo and Holland, 1997). The structural components of the viral capsid are made up of the VP2 protein (Law et al., 2010).

Several molecular approaches have been used to characterize IBDV's antigenic variation (Liu et al., 1994; Jackwood and Sommer-Wagner, 2007). This antigenic drift has been traced to eight amino acid mutations in the capsid protein's HVR, which contains the most valuable genetic data about strain diversity (Heine et al., 1991). The nucleotide and amino acid sequences of the VP2 HVR from each of the five Egyptian isolates showed the greatest homology to the relevant classical and vvIBDV strains. In comparison, the nucleotide sequence analysis of the study vvIBDV isolates and the current Egyptian vvBDV strains indicated varied identities, suggesting that the virus in circulation has genetic diversity, as antigenic alterations have occurred in more recent IBDV viruses. Given that RNA viruses have a high mutation rate due to the RNA polymerase's low proofreading ability, genetic diversity is common (Durairaj et al., 2013).

According to a phylogenetic study, IBDV viruses are categorized into seven different genogroups (G1-G7, Michel and Jackwood, 2017). Based on HVR nucleotide sequences, the two strains under examination (OK092296 and OK092299) were phylogenetically grouped with IBD viruses of genogroup 1 (G1). Based on the mutational study and the existence of specific amino acid residues in the HVR, these two strains were designated as classical IBDVs viruses (G1), which might have been generated from vaccine strains already authorized to be used in Egyptian poultry farms.

The amino acid sequence findings of the research isolates indicated the features of each isolate. Notably, the VP2 gene continues to be the fundamental gene that largely influences the genetic and antigenic characteristics determinant of IBDV, as a result, it is frequently employed for the molecular characterization of virus (Letzel et al., 2007; Brandt et al., 2001). The amino acid residues found in the HVR for the strains OK092296 and OK092299 were the characteristic of classical strains (Table 1). To this end, the strains are likely to share

genetic and antigenic traits with classical strains (vaccine strain).

The P domain is one of the three domains of the VP2 protein and is composed of four sub domains: P_{BC} loop structures, P_{DE}, P_{FG}, and P_{HI}. all of which may be seen on the virion's surface (Coulibaly et al., 2005). The mutation study of the loops of the P domain of the VP2 protein revealed that the strains were related to the vaccination used in the flock (immune complex, 2512 W). However, the flocks were vaccinated with Transmune2512ceva®/ which composed of Immune complex vaccines join the virus to a viral-neutralizing element, preventing it from being neutralized by antibodies derived from the mother. The virus replicates and serves as an immune stimulator when maternally produced antibodies diminish (Babazadeh and Asasi, 2021). The samples were taken between 25 and 35 days after the vaccination. A previous study provided a justification that viral shedding of the vaccine may be detected 15 days after inoculation (Corley et al., 2001).

Present strains (OK092295, OK092297, and OK092298) indicated the amino acid positions (222A, 242I, 256I, 284A, 294I, and 299S, Figure 1) that are characteristic of vvIBDV strains (Van Loon et al., 2002), indicating that these three strains belong to genogroup3. As it is located at the tip of the PBC loop, the amino acid at position 222 is essential. A mutation in the amino acid sequence at position 222 might lead to vaccination failure (Brown et al., 1994). All vvIBDV isolated had no substitutions at position (222) displaying A (Alanin) amino acid in this position. The discovery, in conjunction with the A284T alteration, has been demonstrated to impact vvIBDV cell culture tissue adaptability (Abrams and Kandel, 1988; Lim et al., 1999). Demonstrated the adaption of a vvIBDV (HK46 strain) to chicken embryonic fibroblasts by site-directed mutagenesis of D279N and A284T residues. Another mutagenesis investigation found that Q253H and A284T mutations contributed considerably to vvIBDV tissue adaption (Loon et al., 2002).

In all vvIBDV strains, a unique SspI site on VP2 according to (Jackwood et al. 2011). As a result, this SspI site has been utilized as a genetic marker to predict a highly virulent phenotype, which must be verified *in vivo*. As revealed in the amino acid alignment report, the Ssp I restriction site on VP2 was found in all of the vvIBDV isolates shown to be indicative of vvIBDV strains but not in the classic vaccine strains (Figure 2). However, not all vvIBDV strains have this identifier, and some non-vvIBDV strains have been shown to carry the SspI marker (Sapats and Ignjatovic, 2002).

All research isolates of VP2 HVR preserve the virulence characteristic of serine-rich heptapeptide sequences (SWSASGS), which are located around the primary hydrophilic peak (B, PHI). Furthermore, the strains containing glutamine at position 253 are more pathogenic than those with histidine at position 253. Recently, there has been a lot of focus on amino acids at position 253, where histidine or glutamine might be detected. Due to the prolonged vaccination campaigns conducted in the field using live attenuated viruses, the viruses used may mutate and hence lose their pathogenic potential (Zierenberg et al., 2001).

CONCLUSION

The study concluded that the presence of two isolates similar to vaccine origin with the attendant of three isolates of the vvIBDV in the field indicates dual circulation of both G1 and G3 strains. Moreover, the findings indicated the genetic diversity of the recent IBDV isolated from vaccinated chicken flocks.

DECLARATIONS

Acknowledgments

The authors would like to thank all of the farm owners and veterinarians who assisted with this study. There was no financial support for this study.

Author`s contribution

Zeinab Mossad and Ali Zanaty conceptualized the study. Mahmoud Said and Mohamed Samy participated in performing methodology. Zeinab Mossad was responsible for software applications and investigation. Ali Zanaty and Fatma Amer helped in validation and analysis. Neveen Rabie and Mohamed Soliman were responsible for resources and data curation, and Mohamed Soliman was responsible for writing the original draft. Zeinab Mosaad and Mohamed Soliman wrote and revised the final draft of manuscript. All authors read and approved the published version of the manuscript.

Competing interests

The authors claim that they have no conflicts of interest.

Ethical considerations

These authors investigated ethical concerns such as (plagiarism, misconduct, permission to publish, double publishing, data fabrication and/or falsification, and/or submission, and redundancy).

Funding

There was no funding support for this study.

Consent to publish

The farm owners have provided written informed approval for the publication of this study.

Data availability statement

The data described in this study are accessible from the relevant authors upon request.

REFERENCES

Abdel-Alim G, Awaad MH, and Saif YM (2003). Characterization of Egyptian field strains of infectious bursal disease virus. Avian Diseases, 47(4): 1452-1457. DOI: https://www.doi.org//10.1637/7032

Abrams TW and Kandel ER (1988). Is contiguity detection in classical conditioning a system or a cellular property? Learning in Aplysia suggests a possible molecular site. Trends in Neurosciences, 11(4): 128-135. DOI: https://www.doi.org/10.1016/0166-2236(88)90137-3

Aliyu HB, Hair-Bejo M, Omar AR, and Ideris A (2021). Genetic diversity of recent infectious bursal disease viruses isolated from vaccinated poultry flocks in Malaysia. Frontiers Veterinary Science, 8: 643976. DOI: https://www.doi.org/10.3389/fvets.2021.643976

Babazadeh D and Asasi K (2021). Effects of in *ovo* synbiotic injection on growth performance, intestinal bacterial load and antibody titres in broiler chickens vaccinated against infectious bursal disease. Bulgarian Journal of Veterinary Medicine, 24(4): 520-523. DOI: https://www.doi.org/10.15547/bjvm.2298

Brandt M, Yao K, Liu M, Heckert RA, and Vakharia VN (2001). Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. Journal of Virology, 75(24): 11974-11982. DOI: http://www.doi.org/10.1128/JVI.75.24.11974-11982.2001

Brown MD, Green P, and Skinner MA (1994). VP2 sequences of recent European 'very virulent' isolates of infectious bursal disease virus are closely related to each other but are distinct from those of 'classical'strains. Journal of General Virology, 75(3): 675-680. DOI: https://www.doi.org/10.1099/0022-1317-75-3-675

Corley MM, Giambrone JJ, and Dormitorio TV (2001). Detection of infectious bursal disease vaccine viruses in lymphoid tissues after in *ovo* vaccination of specific-pathogen-free embryos. Avian Diseases, 45(4): 897-905. Available at: https://www.jstor.org/stable/1592869

Coulibaly F, Chevalier C, Gutsche I, Pous J, Navaza J, Bressanelli S, Delmas B, and Rey FA (2005). The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell, 120(6): 761-772. DOI: https://www.doi.org/10.1016/j.cell.2005.01.009

Domingo E and Holland JJ (1997). RNA virus mutations and fitness for survival. Annual Review of Microbiology, 51(1): 151-178. DOI: http://www.doi.org/10.1146/annurev.micro.51.1.151

Dufour-Zavala L (2008). A laboratory manual for the isolation, identification, and characterization of avian pathogens. American Association of Avian Pathologists. Available at: https://www.semanticscholar.org/paper/A-laboratory-manual-for-the-isolation%2C-and-of-avian-Dufour-Zavala/ac7cf3149766809f9da31b28622f4b0659320baa#paper-header

Durairaj V, Linnemann E, Icard AH, Williams SM, Sellers HS, and Mundt E (2013). An in *vivo* experimental model to determine antigenic variations among infectious bursal disease viruses. Avian Pathology, 42(4): 309-315. DOI: http://www.doi.org/https://doi.org/10.1080/03079457.2013.793783

El-Batrawi AM (1990). Studies on severe outbreaks of in fectious bursal disease. 1. The natural and experimental disease, proceedings second scientific conference of the egyptian veterinary poultry association. pp. 239-252. Availabale at: https://cir.nii.ac.jp/crid/1583950399224505856

Eladl AH, Mosad SM, El-Shafei RA, Saleh RM, Ali HS, Badawy BM, and Elshal MF (2020). Immunostimulant effect of a mixed herbal extract on infectious bursal disease virus (IBDV) vaccinated chickens in the context of a co-infection model of avian influenza virus H9N2 and IBDV. Comparative Immunology, Microbiology and Infectious Diseases, 72: 101505. DOI: http://www.doi.org/10.1016/j.cimid.2020.101505

Eterradossi N and Saif YM (2013). Infectious bursal disease. Diseases of Poultry, 4: 219-246. DOI: https://www.doi.org/10.1002/9781119421481.ch7

Fan L, Wang Y, Jiang N, Gao L, Li K, Gao Y, Cui H, Pan Q, Liu C, Zhang Y et al. (2020). A reassortment vaccine candidate of the novel variant infectious bursal disease virus. Veterinary Microbiology, 251: 108905. DOI: https://www.doi.org/10.1016/j.vetmic.2020.108905

Heine HG, Haritou M, Failla P, Fahey K, and Azad A (1991). Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains. Journal of General Virology, 72(8): 1835-1843. DOI: https://www.doi.org/10.1099/0022-1317-72-8-1835

Hussain I, Rasool M, and Mahmood M (2004). Production of hyperimmune serum against infectious bursal disease virus in rabbits. Pakistan Veterinary Journal, 24(4): 179-183. Available at: http://pvj.com.pk/pdf-files/24_4/179-183.pdf

Islam M, Rahman S, Noor M, Chowdhury E, and Müller H (2012). Differentiation of infectious bursal disease virus (IBDV) genome segment B of very virulent and classical lineage by RT-PCR amplification and restriction enzyme analysis. Archives of Virology, 157(2): 333-336. DOI: https://www.doi.org/10.1007/s00705-011-1159-9

Jackwood D, Sreedevi B, LeFever L, and Sommer-Wagner S (2008). Studies on naturally occurring infectious bursal disease viruses suggest that a single amino acid substitution at position 253 in VP2 increases pathogenicity. Virology, 377(1): 110-116. DOI: https://www.doi.org/10.1016/j.virol.2008.04.018

Jackwood DJ and Sommer-Wagner SE (2007). Genetic characteristics of infectious bursal disease viruses from four continents. Virology, 365(2): 369-375. DOI: https://www.doi.org/10.1016/j.virol.2007.03.046

Jackwood DJ and Sommer-Wagner SE (2011). Amino acids contributing to antigenic drift in the infectious bursal disease Birnavirus (IBDV). Virology, 409(1): 33-37. DOI: http://www.doi.org/10.1016/j.virol.2010.09.030

Jackwood DJ, Sommer-Wagner SE, Crossley BM, Stoute ST, Woolcock PR, and Charlton BR (2011). Identification and pathogenicity of a

natural reassortant between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV. Virology, 420(2): 98-105. DOI: https://www.doi.org/10.1016/j.virol.2011.08.023

Khan RSA, Sajid S, Habib M, Ali W, Salah-Ud-Din Shah M, and Sarfraz M (2017). History of Gumboro (infectious bursal disease) in Pakistan. Saudi Pharmaceutical Journal, 25(4): 453-459. DOI: https://www.doi.org/10.1016/j.jsps.2017.04.005

Law RH, Lukoyanova N, Voskoboinik I, Caradoc-Davies TT, Baran K, Dunstone MA, D'Angelo ME, Orlova EV, Coulibaly F, Verschoor S et al. (2010). The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature, 468(7322): 447-451. DOI: http://www.doi.org/10.1038/nature09518

Letzel T, Coulibaly F, Rey FA, Delmas B, Jagt E, van Loon AA, and Mundt E (2007). Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease. virus. Journal of Virology, 81(23): 12827-12835. DOI: https://www.doi.org/10.1128/JVI.01501-07

Lim BL, Cao Y, Yu T, and Mo CW (1999). Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2. Journal of Virology, 73(4): 2854-2862. DOI: https://www.doi.org/10.1128/JVI.73.4.2854-2862.1999

Liu HJ, Giambrone J, and Dormitorio T (1994). Detection of genetic variations in serotype I isolates of infectious bursal disease virus using polymerase chain reaction and restriction endonuclease analysis. Journal of Virological Methods, 48(2-3): 281-291. DOI: https://www.doi.org/10.1016/0166-0934(94)90127-9

Loon Av, Haas Nd, Zeyda I, and Mundt E (2002). Animal-RNA viruses-Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. Journal of General Virology, 83(1): 121-130. DOI: https://www.doi.org/10.1099/0022-1317-83-1-121

Mawgod SA, Arafa AS, and Hussein HA (2014). Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from broiler flocks in Egypt. International Journal of Veterinary Science and Medicine, 2(1): 46-52. DOI: https://www.doi.org/10.1016/j.ijvsm.2014.02.004

Metwally A, Yousif A, Shaheed I, Mohammed W, Samy A, and Reda I (2009). Re-emergence of very virulent IBDV in Egypt. International Journal of Virology, 5(1): 1-17. DOI: https://www.doi.org/10.3923/ijv.2009.1.17

Michel LO and Jackwood DJ (2017). Classification of infectious bursal disease virus into genogroups. Archives of Virology, 162(12): 3661-3670. Available at: https://link.springer.com/article/10.1007/s00705-017-3500-4

Mohamed MA, Elzanaty KES, Bakhit BM, and Safwat MM (2014). Genetic characterization of infectious bursal disease viruses associated with gumboro outbreaks in commercial broilers from asyut Province, Egypt. International Scholarly Research Notices, 2014: Article ID 916412. DOI: http://www.doi.org/10.1155/2014/916412

Ray SM, Ashash U, and Muthukumar S (2021). A field study on the evaluation of day-of-hatch and in grow-out application of live infectious bursal disease virus vaccine in broiler chickens. Poultry Science, 100(8): 101252. DOI: https://www.doi.org/10.1016/j.psj.2021.101252

Sajid S, Rahman SU, Mohsin Gilani M, Sindhu ZUD, Ali MB, Hedfi A, Almalki M, and Mahmood S (2021). Molecular characterization and demographic study on infectious bursal disease virus in faisalabad district. PLoS One, 16(8): e0254605. DOI: https://www.doi.org/10.1371/journal.pone.0254605

Sapats SI and Ignjatovic J (2002). Restriction fragment length polymorphism analysis of the VP2 gene of Australian strains of infectious bursal disease virus. Avian Pathology, 31(6): 559-566. DoI: https://www.doi.org/10.1080/0307945021000024625

Shabbir MZ, Ali M, Abbas M, Chaudhry UN, Zia Ur R, and Munir M (2016). Molecular characterization of infectious bursal disease viruses from Pakistan. Archieve of Virology, 161(7): 2001-2006. DOI: https://www.doi.org/10.1007/s00705-016-2869-9

Shehata AA, Sultan H, Halami MY, Talaat S, and Vahlenkamp TW (2017). Molecular characterization of very virulent infectious bursal disease virus strains circulating in Egypt from 2003 to 2014. Archives of Virology, 162(12): 3803-3815. DOI: https://www.doi.org/10.1007/s00705-017-3554-3

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molcular Biolology and Evolution, 30(12): 2725-2729. DOI: https://www.doi.org/10.1093/molbev/mst197

van den Berg TP, Morales D, Eterradossi N, Rivallan G, Toquin D, Raue R, Zierenberg K, Zhang MF, Zhu YP, Wang CQ et al. (2004). Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. Avian Pathology, 33(5): 470-476. DOI: http://www.doi.org/10.1080/03079450400003650

Van Loon A, De Haas N, Zeyda I, and Mundt E (2002). Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. Journal of General Virology, 83(1): 121-129. DOI: https://www.doi.org/10.1099/0022-1317-83-1-121

Vukea PR, Willows-Munro S, Horner RF, and Coetzer TH (2014). Phylogenetic analysis of the polyprotein coding region of an infectious South African bursal disease virus (IBDV) strain. Infecttion, Genetetics and Evoloution, 21: 279-286. DOI: http://www.doi.org/10.1016/j.meegid.2013.11.017

Yovel Y, Franz MO, Stilz P, and Schnitzler HU (2008). Plant classification from bat-like echolocation signals. PLoS Computational Biology, 4(3): e1000032. DOI: https://www.doi.org/10.1371/journal.pcbi.1000032

Zierenberg K, Raue R, and Müller H (2001). Rapid identification of" very virulent" strains of infectious bursal disease virus by reverse transcription-polymerase chain reaction combined with restriction enzyme analysis. Avian Pathology, 30(1): 55-62. DOI: http:// https://www.doi.org/10.1080/03079450020023203

Instructions for Authors

SUBMIT AN ARTICLE

Manuscript as Original Research Paper, Short Communication, Case Reports and Review or Mini-Review are invited for rapid peer-review publishing in *the Journal of World's Poultry Research*. Considered subject areas include: Husbandry and management; construction, environment and welfare; exotic and wild birds; Biochemistry and cellular biology; immunology, avian disease control; layer and quail management; nutrition and feeding; physiology, genetics, reproduction and hatching; technology, processing and food safety... view full aims and scope

- JWPR EndNote Style
- Manuscript Template (MS Word)
- Sample Articles
- Declaration form
- Publication Ethics

Submission

The manuscript and other correspondence should preferentially be submit online. Please embed all figures and tables in the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and will send an email regarding your submission. Meanwhile, the authors can submit or track articles via editor@jwpr.science-line.com or editorjwpr@gmail.com. All manuscripts must be checked (by English native speaker) and submitted in English for evaluation (in totally confidential and impartial way).

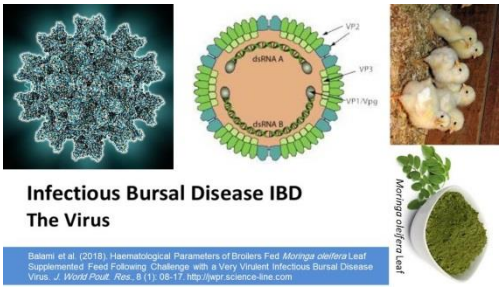
Supplementary information:
The online submission form allows supplementary information to be submitted together with the main manuscript file and covering letter. If you have more than one supplementary files, you can submit the extra ones by email after the initial submission. Author guidelines are specific for each journal. Our Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of journal. If you believe your article is fully edited per journal style, please use our MS Word template before submission.

Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that:
1.The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2.All authors have approved the submission and have obtained permission for publish work.
3.Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the *'Guidelines for the Treatment of Animals in Research and Teaching*'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics Committee Approval
Experimental research involving animals should have been approved by author's institutional review board or ethics committee. This information can be mentioned in the manuscript including the name of the board/committee that gave the approval. The use of animals in experiments will have observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Graphical Abstract
Authors should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) x 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi.
Note: Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication. For preparing a Professional Graphical Abstract, please click **here**.



Presentation of the article

Main Format

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 17pt in capitalization for the title, 10pt for the section headings in the body of the text and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. Abbreviations in the article title are not allowed.
Manuscripts should be arranged in the following order:
1. TITLE (brief, attractive and targeted);

2. Name(s) and Affiliation(s) of author(s) (including post code) and corresponding E-mail; ORCID: 0000-0000-0000-0000
3. ABSTRACT
4. Key words (separate by semicolons; or comma,)
5. Abbreviations (used in the manuscript)
6. INTRODUCTION
7. MATERIALS AND METHODS
8. RESULTS
9. DISCUSSION
10. CONCLUSION
11. DECLARATIONS
12. REFERENCES
13. Tables
14. Figure captions
15. Figures

Results and Discussion can be presented jointly.
Discussion and Conclusion can be presented jointly.

Article Sections Format

Title should be a brief phrase describing the contents of the paper. The first letter of each word in title should use upper case. The Title Page should include the author(s)'s full names and affiliations, the name of the corresponding author along with phone and e-mail information. Present address(es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 350 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 8 key words that will provide indexing references should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail. The ethical approval for using animals in the researches should be indicated in this section with a separated title.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion should be brief and tight about the importance of the work or suggest the potential applications and extensions. This section should not be similar to the Abstract content.

Declarations including Ethics, Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are necessary.

Acknowledgments of persons, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

Declarations section - Please include declarations heading

Please ensure that the sections:
-Ethics (and consent to participate)
-Consent to publish
-Competing interests
-Authors' contributions
-Availability of data and materials
are included at the end of your manuscript in a Declarations section.

Consent to Publish
Please include a 'Consent for publication' section in your manuscript. If your manuscript contains any individual person's data in any form (including individual details, images or videos), consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish. You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication). If your manuscript does not contain any individual persons data, please state "Not applicable" in this section.

Authors' Contributions
For manuscripts with more than one author, JWPR require an Authors' Contributions section to be placed after the Competing Interests section.
An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
For authors that equally participated in a study please write 'All/Both authors contributed equally to this work.' Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests
Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.".
Journal World's Poultry Research adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship
We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in sequence of author name. We have this policy to prevent the fraud.

Acknowledgements
We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.
Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data Deposition
Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

References:

A JWPR reference style for **EndNote** may be found **here**.

1. All references to publications made in the text should be presented in a list with their full bibliographical description. DOI number or the link of article should be added to the end of the each reference.
2. In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's surname should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.
3. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b, etc.
4. Names of authors and title of journals, published in non-latin alphabets should be transliterated in English.
5. A sample of standard reference is "1th Author surname A, 2th Author surname B and 3th Author surname C (2013). Article title should be regular and 9 pt. Journal of World`s Poultry Research, Volume No. (Issue No.): 00-00." DOI:XXX."
6. Journal titles should be full in references. The titles should not be italic.
7. References with more than 10 authors should list the first 10 authors followed by 'et al.'
8. The color of references in the text of article is blue. Example: (Preziosi et al., 2002; Mills et al., 2015).

9. At least 35% of the references of any submitted manuscript (for all types of article) should include scientific results published in the last five years.

-Examples (at the text- blue highlighted)

Abayomi (2000), Agindotan et al. (2003), Vahdatpour and Babazadeh (2016), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993, 1995), (Kumasi et al., 2001).

--Examples (at References section)

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development. pp 31-38. DOI:XXX.

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology, 7: 3535-3539. DOI:XXX.

Tahir Khan M, Bhutto ZA, Abbas Raza SH, Saeed M, Arain MA, Arif M, Fazlani SA, Ishfaq M, Siyal FA, Jalili M et al. (2016). Supplementation of different level of deep stacked broiler litter as a source of total mixed ration on digestibility in sheep and their effects on growth performance. Journal of World`ˢ Poultry Research, 6(2): 73-83. DOI: XXX

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine 13: 191-199.

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27.

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg).

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

Nomenclature and Abbreviations:

Nomenclature should follow that given in NCBI web page and Chemical Abstracts. Standard abbreviations are preferable. If a new abbreviation is used, it should be defined at its first usage. Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution; ...

Abbreviations of units should conform with those shown below:

Decilitre	dl	**Kilogram**	kg
Milligram	mg	**hours**	h
Micrometer	mm	**Minutes**	min
Molar	mol/L	**Mililitre**	ml
Percent	%		

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (the Royal Society of Medicine London 1977).
Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

Formulae, numbers and symbols:

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter 0, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
9. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Xχ - chi, Ψψ - psi, Ωω - omega.

Review/Decisions/Processing

Firstly, all manuscripts will be checked by Docol©c, a plagiarism finding tool. A single blind reviewing model is used by JWPR for non-plagiarized papers. The manuscript is edited and reviewed by the English language editor and three reviewers selected by section editor of JWPR respectively. Also, a reviewer result form is filled by reviewer to guide authors. Possible decisions are: accept as is, minor revision, major revision, or reject. See sample of evaluation form. Authors should submit back their revisions within 14 days in the case of minor revision, or 30 days in the case of major revision.

To submit a revision please click here, fill out the form, and mark "Revised", mention the article code (for example JWPR-1105), attach the revision (MS word) and continue submission. After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be hold responsible for any mistakes shown in the final publication. Manuscripts with significant results are typically reviewed and published at the highest priority.

Plagiarism
There is a zero-tolerance policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts are screened for plagiarism by Docol©c a plagiarism finding tool, before or during publication, and if found they will be rejected at any stage of processing. See sample of Docol©c-Report.

Declaration
After manuscript accepted for publication, a declaration form will be sent to the corresponding author who that is responsible to coauthors' agreements to publication of submitted work in JWPR after any amendments arising from the peer review.

Date of issue
The journal will be issued on 25th of March, June, September and December, each year.

Publication charges
No peer-reviewing charges are required. However, the publication costs are covered through article processing charges (APCs). There is a modest APC of 150 Euro(€) editor fee for the processing of each primary accepted paper (1000-4000 words) to encourage high-quality submissions. APCs are only charged for articles that pass the pre-publication checks and are published. A surcharge will be placed on any article that is over 4000 words in length to cover the considerable additional processing costs. Payment can be made by credit card, bank transfer, money order or check. Instruction for payment is sent during publication process as soon as manuscript is accepted. Meanwhile, this journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charges.

WORD COUNT	PRICE*
1000-4000 words	€150
over 4000 words	€230

* The prices are valid until 30th December 2022.

The Waiver policy
The publication fee will be waived for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *Journal of World's Poultry Research*. The Journal will consider requests to waive the fee for cases of financial hardship (for high quality manuscripts and upon acceptance for publication). Requests for waiver of the submission fee must be submitted via individual cover letter by the corresponding author and cosigned by an appropriate institutional official to verify that no institutional or grant funds are available for the payment of the fee. Letters including the manuscript title and manuscript ID number should be sent to: editor [at] jwpr.science-line.com. It is expected that waiver requests will be processed and authors will be notified within two business day.

The OA policy
Journal of World's Poultry Research is an open access journal which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the *BOAI definition of Open Access*.

Scienceline Language Editing Services

We suggest that authors whose first language is not English have their manuscripts checked by a native English speaker before submission. This is optional, but will help to ensure that any submissions that reach peer review can be judged exclusively on academic merit. We offer a Scienceline service, and suggest that authors contact as appropriate. Please note that use of language editing services is voluntary, and at the author's own expense. Use of these services does not guarantee that the manuscript will be accepted for publication, nor does it restrict the author to submitting to Scienceline journals. You can send the article/s to the following Email: daryoushbabazadeh@gmail.com

Paper Submission Flow

Submission Preparation Checklist

Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines.
The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
The submission file is in Microsoft Word, RTF, or PDF document file format.
Where available, URLs for the references have been provided.
The text is single-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

(Revised on 04 September 2022)

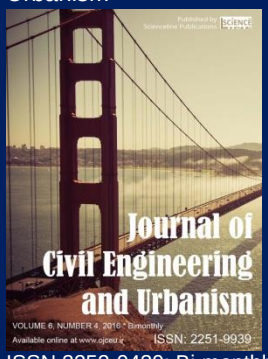
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd is a limited liability non-profit non-stock corporation incorporated in Turkey (Company No. 0757086921600001). Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal | Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism

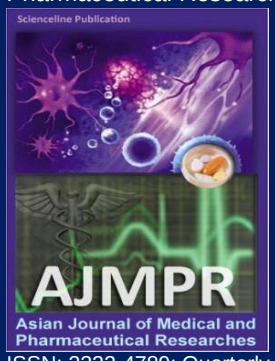


ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

Journal of Life Sciences and Biomedicine

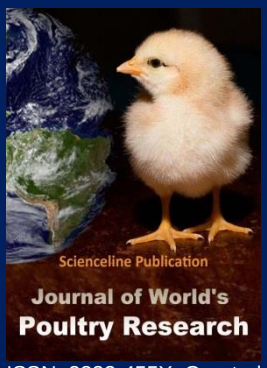
ISSN: 2251-9939; Bi-monthly
View Journal | Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

Asian Journal of Medical and Pharmaceutical Researches



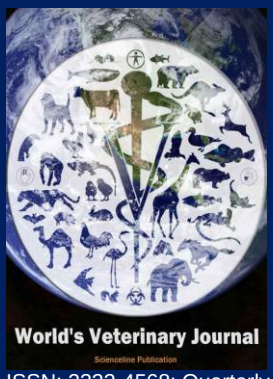
ISSN: 2322-4789; Quarterly
View Journal | Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of World's Poultry Research



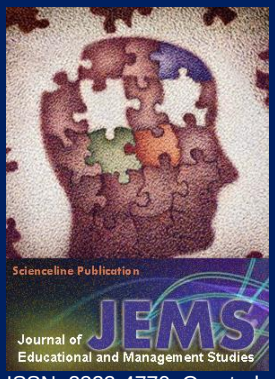
ISSN: 2322-455X; Quarterly
View Journal | Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

World's Veterinary Journal



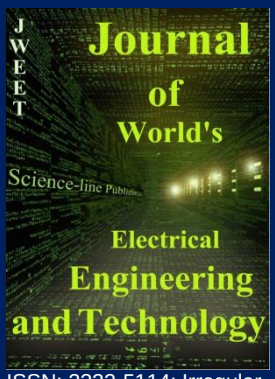
ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of Educational and Management Studies



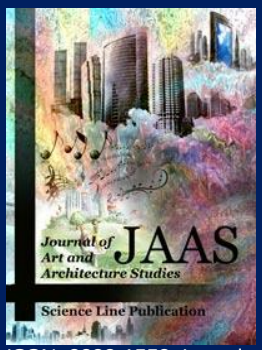
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal | Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Irregular
View Journal | Editorial Board
Email: jaas@science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal | Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal | Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal | Editorial Board
Email: sjmie@science-line.com
Submit Online >>

Scienceline is a non-profit organisation inspired by research funders and led by scholars. Our mission is to help researchers accelerate discovery and innovation by operating a platform for research communication that encourages and recognises the most responsible behaviours in science.

Scienceline Publications, Ltd is a limited liability non-profit non-stock corporation registered in the State of Erzurum, Turkey, with company number 0757086921600001, and branch number 18677/25379 at the address: Scienceline Publications, Ltd., Ömer Nasuhi Bilmen Road, Dönmez Apart., G1/6, Yakutiye, Erzurum 25100, Turkey