In the Name of God

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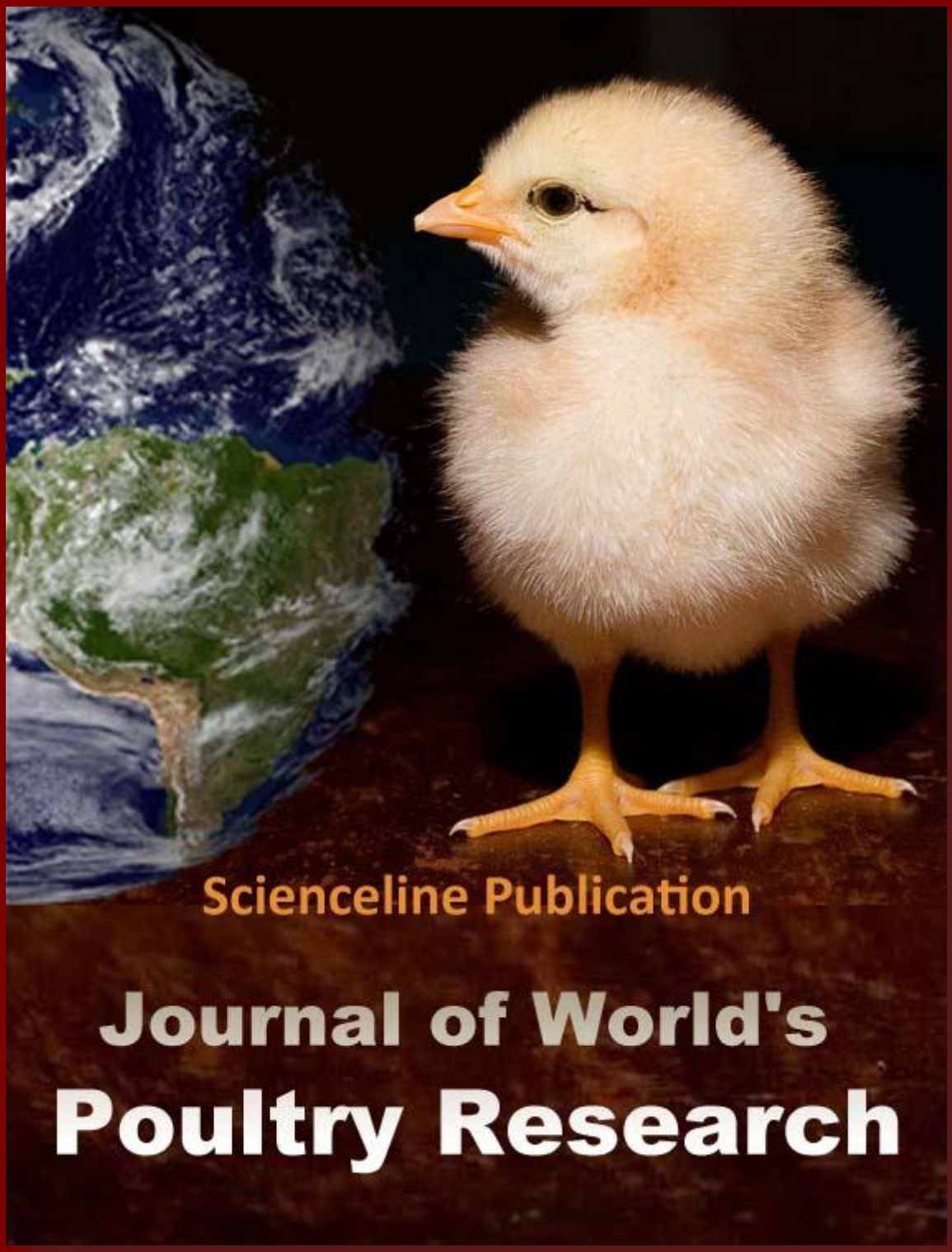

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considered more resistant to endotoxin and information on inflammation and homeostasis in birds supplemented with higher dose of vitamin D_3 when suffer endotoxic shock is lacking. The present study was conducted to compare the effect on hemoglobin concentration and biochemical parameters of broiler chicks by administering toxic dose of vitamin D_3 for 21 days concomitantly challenged with endotoxin. The chicks were randomly divided into four groups viz. A, B, C and D. Hemoglobin concentrations of control groups (A and B) and treatment groups (C and D) did not differ significantly ($P< 0.05$). Hypercalcemia and hyperphosphatemia was observed in both treatment groups in comparison to the control group. No significant ($P< 0.05$) change was observed in the concentrations of total protein and albumin and in the activity of plasma Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase on day 28 of control (A and B) and treatment (C and D) groups. Vitamin D_3 supplementation causes immunomodulation; hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.
Keywords: Broiler chicks**,** Hypercalcaemia, Hypervitaminosis D_3, Hyperphosphatemia

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Research Paper

Effect of Combination of Encapsulated Black Cincau Leaves (*Mesona Palustris* Bl) and Probiotics on Production Performances, Yolk Cholesterol Content and Ammonia Level of Laying Hen.

Natsir MH, Sjofjan O, Ardiansah I, Khairani S and Elliyana (2018). **Effect of Combination of Encapsulated Black Cincau Leaves (*Mesona Palustris* Bl) and Probiotics on Production Performances, Yolk Cholesterol Content and Ammonia Level of Laying Hen.** *J. World Poult. Res.*, 8 (4): 105-110. http://jwpr.science-line.com

Natsir MH, Sjofjan O, Ardiansah I, Khairani S and Elliyana.

J. World Poult. Res. 8(4): 105-110, 2018; pii: S2322455X1800015-8

ABSTRACT
The purpose of this research was to determine addition of natural feed additives from combination of encapsulated black cincau leaves and probiotics on feed intake, feed conversion, hen day production (HDP), egg mass, income over feed cost (IOFC), egg weight, yolk cholesterol content and ammonia levels in excreta. One hundred ninety-two laying hens at 28 weeks were used in this experiment. Egg mass which used before this research was 64.63±2.97 g/ day with CV was 4.59%. The method which used was experimental of completely randomized design (CRD) with four treatments and six replications (eight-layers each). The treatments used were T0: basal feed; T1: basal feed + combination of encapsulated black cincau leaves and probiotics 0.5%; T2: basal feed + combination of encapsulated black cincau leaves and probiotics 1%; T3: basal feed + combination of encapsulated black cincau leaves and probiotics 1.5%. Data were analyzed by using analysis of variance, if any significant effect, it would be further tested by Duncan's Multiple Range Test. The result showed that no siginficant effect ($P> 0.05$) on feed intake, feed conversion, HDP, egg mass, IOFC, egg weight and yolk cholesterol content, but any significant effect ($P< 0.05$) on ammonia level. This research concludes that using 1.5% of combination of encapsulated black cincau leaves and probiotics give better result than others.
Keywords: Black cincau leaves, Egg quality, Encapsulated probiotic, Hen production, Laying hen

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Research Paper

Comparison of Three Lines of Japanese Quails Revealed a Remarkable Role of Plumage Color in the Productivity Performance Determination.

Rasol Al-Kafajy F, Sahib Al-Shuhaib MB, Salah Al-Jashami Gh, Mohammed Al-Thuwaini T (2018). **Comparison of Three Lines of Japanese Quails Revealed a Remarkable Role of Plumage Color in the Productivity Performance Determination.** *J. World Poult. Res.*, 8 (4): 111-119. http://jwpr.science-line.com

Rasol Al-Kafajy F, Sahib Al-Shuhaib MB, Salah Al-Jashami Gh, Mohammed Al-Thuwaini T.

J. World Poult. Res. 8(4): 111-119, 2018; pii: S2322455X1800016-8

ABSTRACT
The study was conducted to compare body weight, egg, and carcass characteristics, as well as several biochemical parameters amongst three lines of plumage color of quails, including, black, white, and brown (n= 200 each). Body weight was analyzed on a weekly basis throughout the study period (third– 13th week of age). Eggs were collected for seven consecutive weeks of sexual maturity (seventh – 13th week of age). In addition to egg quality measurements, 16 serum biochemical parameters were also determined. The brown line had exerted significantly higher values of body weight in most analyzed weeks of sexual maturity. It had given higher values of albumen height and shell thickness, as well as carcass dressing than other lines. Simultaneously, a significantly high number of eggs in the white line were observed in the most analyzed weeks. Besides, it had given higher values in terms of shell and yolk weights, as well as several carcass characteristics, such as the heart, thigh, breast, and back. The biochemical analyses had shown no significant differences amongst the analyzed populations with exception of a higher concentration of amylase in the brown line. In conclusion, our study revealed the presence of a clear superiority of the brown and white lines in terms of the meat and egg productivity, respectively. Therefore, we recommend breeders to raise brown and white lines for a better production of meat and eggs, respectively, whereas the black line has shown the least productive characteristics than other two lines throughout the study period.
Keywords: Eggs, Japanese quails, Line, Meat, Production, Serum

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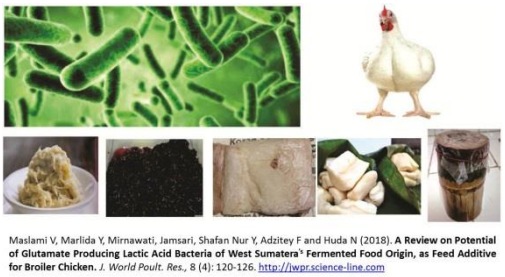
Review

A Review on Potential of Glutamate Producing Lactic Acid Bacteria of West Sumatera's Fermented Food Origin, as Feed Additive for Broiler Chicken.



Maslami V, Marlida Y, Mirnawati, Jamsari, Shafan Nur Y, Adzitey F and Huda N.

J. World Poult. Res. 8(4): 120-126, 2018; pii: S2322455X1800017-8

ABSTRACT
Increasing broiler populations must be supported by cheap and high quality feed. Improving the quality of feed can be done by adding feed additives. Glutamate is a non-essential amino acid that can be used as a feed additive in the form of flavoring agents in broiler feed which functions as a neurotransmitter of taste, basic structure of proteins, and in metabolism of the body. Lactic Acid Bacteria (LAB) are one of the microbes that are considered faster and safe in producing glutamate. Fermented foods of West Sumatera, Indonesia origin serve as sources of LAB include dadih (fermented milk), asam durian (fermented durian), ikan budu (fermented fish) and tapai (fermented rice and cassava). The West Sumatra's fermented foods are potential sources of glutamate. Supplementation of glutamate in broiler diet can increase body weight, protein digestibility, reduce faecal ammonia and improve carcass quality (improve umami taste, and reduce bruises and abdominal fat).
Keywords: Carcass quality, Feed additive, Fermented food, Glutamate, Lactic acid bacteria, Performance

[Full text-PDF] [XML]

Research Paper

Molecular Survey and Characterization of H5N8 Isolates during 2016-2017 on Egypt.

Sedeik M El-S, Elshal NA, Awad AM and Kandil N.

J. World Poult. Res. 8(4): 127-133, 2018; pii: S2322455X1800018-8

ABSTRACT
Avian influenza (AI) disease still threat poultry industry in Egypt causing great economic losses. In order to identify and characterize the agent of suggestive clinical cases of AI disease, 28 flocks showing clinical signs suspected to be due to AI infections have been investigated. By slide Haemagglutination (HA), the positive samples were 14/28 and concerning the results of real time- reverse transcriptase polymerase chain reaction (RRT-PCR), 2/14 samples were positive to AI H5, 7/14 to New castle disease virus (NDV), 1/14 to H9 and 4/14 co-infected (2 samples had NDV + AI H5 and others had NDV + AI H9). These positive PCR samples were subjected to further characterization by genotyping and sequencing analysis. The two isolated of H5 AI strain were classified to H5N8 which, related to Russian strains (clade 2.3.4.4) and the genetic analysis approved little relationship between these two H5N8 strain and the commercial AI vaccines with percent (80- 91.7%). So, the researchers should have more monitoring for these viral diseases with effective biosecurity and quarantine measures to minimize the disease occurrence.
Key words: Avian influenza, flocks, molecular, survey

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Homepage: www.science-line.com
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Emails:
administrator@science-line.com
saeid.azar@atauni.edu.tr

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Review Paper, PII: S2322455X1800012-8

Survey of Highly Pathogenic Avian Influenza Virus (H5N1) and Its Reoccurring Threat: A Brief Review on Different Quails Worldwide

Khushboo Arya[1], Roshani Gupta[2] and Vijay Laxmi Saxena[1]*

[1]*Bioinformatics Centre of Department of Biotechnology, Department of Zoology Dayanand girl's college Kanpur, India*
[2]*MRD Life Sciences™ Lucknow-226010, India*

*Corresponding author's Email: *vlaxmisaxena@gmail.com*

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ABSTRACT

This Review aims to understand the present status of influenza viruses and its epidemiology. The first case in India has been reported in the Dasarahalli village near Bangalore after six months of India's declaration that it is free from H5N1 and H5N8 from world organization for animal health. The recent controversy regarding outbreaks and cross-species barrier resulted in highly contagious infection with fatal outcomes, triggered menace all over India with remarkable economic consequences. Thus, we had reviewed epidemiology, virology, surveillance, transmission, detection, treatment and associated control measures to depict the current perspective of Influenza epidemic. We also studied different Quails and its comprehensive portal susceptible to influenza and in-depth genetic characterization of virus due to new viral mutant causing host-virus complications, virus mutation, and vaccination with its prompt administration as it is the urgency of the era. Addressing aspects of the epidemiology of the H5N1 and drug resistance genomic signatures infecting poultry and Humans helps to frontier our ability to minimize data gaps and maximize the better results of the available H5N1 studies.

Keywords: H5N1, Avian influenza viruses, Quail, Transmission, Detection

INTRODUCTION

The first avian influenza infection was recorded in Nandurbar and Jalgaon districts in Maharashtra, India during February 2006. The villagers were isolated from infected places and blood samples from 150 people showing symptoms of infection were sent to the National Institute of Virology, Pune, India (Mascarenhas, 2018). An episode of the exceedingly pathogenic avian influenza strain H5N8, which spread through Asia, Europe and the Middle East over the most recent years, has been accounted in India, as per the World Organization for Animal Health (OIE, Office International des Epizooties). Recently, India has reported in excess of 900 birds after it revealed an instance of approximately 942 birds at Humnabad, Bidar district of Karnataka, said by Karnataka health and family welfare department and its associated officials in India (Aiyappa, 2016). There has been no further outbreak reported in the country thereafter. But soon after it had reported outbreaks of highly pathogenic Avian Influenza Viruses (AVI) at various epicenters in Delhi, Gwalior (MP), Rajpura (Punjab), Hissar (Haryana), Bellary (Karnataka), Allappuzha and Kottayam (Kerala), Ahmedabad (Gujarat), Daman (Daman), Khordha and Angul (Odisha) during October, 2016 to February, 2017. India declared itself free from avian influenza (H5N8 and H5N1) from 6th June, 2017 and notified the same to OIE (Mohan, 2017). Once again, an outbreak of the highly contagious H5N8 bird flu virus that popped up in poultry birds in Dasarahalli village near Bangalore, after six months, India declared itself free of this virus by OIE (Mudur, 2018). Recently detected H5N8 is a mutant of earlier H5N1 and H7N7 viruses said by Department of Neurovirology, at the National Institute of Mental Health and Neurosciences (NIMHANS). The episode is a reoccurrence of the H5N8 avian influenza strain. The infection was detected after nine birds died out of 951 susceptible birds. The remaining 942 birds were killed as

reported by OIE. Therefore, a wellspring of the episode stays uncertain at this stage, for recent worldwide look up, Saudi Arabia's capital city, Riyadh, has effectively detailed four instances of the avian strain H5N8. Iraq was compelled to win now 43000 chickens, following episodes of the same avian flu strain. Also, the Russian military was brought in to Kostroma Oblast to manage the result of biggest H5N8 avian influenza flare-up in the district, which culled six lakh chickens (Vorotinkov, 2018).

Figure 1. Highly pathogenic avian influenza viruses outbreaks as reported in start of 2018 in domestic bird's subtypes of different regions like Asia: H5N1, H5N2, H5N3, H5N6, H5N8, H7N9; Africa: H5N1, H5N2, H5N8; Europe: H5N1, H5N2, H5N5, H5N6, H5N8, H5N9, H7N7; America: H5N1, H5N2, H5N8, H7N3, H7N8, H7N9; Oceania: H7N2 (world organization for animal health, 2018)

Epidemiology and Genotypic characteristics of the influenza virus

Eastern region of India has experienced continual outbreaks of H5N1 HPAI virus since February 2006 particularly in the states of Maharashtra, Gujarat and Madhya Pradesh. Subsequently, the outbreaks of the HPAI were reported from the states Manipur, Assam, Sikkim and West Bengal (Murugkar et al., 2008). Since then repeated outbreaks of the HPAI have been reported in the states West Bengal, Assam and Tripura (Nagarajan et al., 2009; Nagarajan et al., 2012)

Aquatic wild birds are the natural reservoir of influenza viruses (Slemons, et al., 1974, Hinshaw, et al., 1980, Webster, et al., 1992, Alexander, 2000). AIV predominantly replicate in the gastrointestinal and respiratory tracts of the Quails and are excreted from the oral cavity, nostrils, conjunctiva, and cloaca of infected birds (Swayne, et al., 2008). AIV belongs to the family *Orthomyxoviridae* and the genus influenza virus A (Palese, 2007). These have negative sense, single stranded and segmented RNA genome with eight segments of gene encoding for at least ten Proteins Namely Polymerase Basic 1 (PB1), PB2, Matrix 1 (M), M2, Polymerase Acid (PA), Haemagglutinin (HA), Nucleoprotein (NP), Neuraminidase (NA), Non-Structural (NS1and NS2). These NA and HA have a role in virus infectivity and type of surface glycoproteins. The HA protein attaches to the host cell with the help of virus and also a significant target of the humoral immune responses (Chen et al., 2018). NA removes sialic acid from glycoproteins and thus helps to increase and spread of progeny virions. Low Pathogenic Avian Influenza Viruses (LPAIVs) contain an HA cleavage site and can be cleaved by proteases which resides in intestinal and respiratory tracts (Alexander, 2007). Highly Pathogenic Avian Influenza Virus (HPAIVs) causes systematic infection and high mortality in chickens and other terrestrial poultry like ducks,waterfoels in correspondence to their subtype of HPAIVs. Two subtypes namely H5 and H7 of LPAIVs can naturally switch to a highly pathogenic phenotype by the process of spontaneous acquisition of a multibasic cleavage site during circulation in poultry (Kawaoka et al., 1985; Webster et al., 1989; Horimoto et al., 1995). Avian and Human viruses preferentially bind to sialic acid linked to galactose via an α2-3 linkage (SAα2, 3Gal) and galactose via an α2-6 linkage (SAα2, 6Gal), respectively (Kumlin et al., 2008). The Receptor Binding Site (RBS) is located at the distal membrane end of each HA monomer and essential amino acids for host determinant are in the residues 222 and 224 in the HA of H5 (equivalent to residues 226 and 228 in the HA of H2 and H3 (Kawaoka et al., 1985; Webster et al., 1989; Connor et al., 1994; Horimoto et al., 1995). Avian influenza viruses H5N1 (Yuen et al., 1998), H9N2 (Cheng, et al., 2011), H7N7 (Banks, et al., 1998), H7N3 (Tweed et al., 2004) and H10N7 (Arzey et al., 2012) had jumped species barriers and caused Human infection. Among these, H5N1 exhibits the property of most virulence, having mortality rate of approx. Sixty percent causing havoc situation worldwide. Influenza viruses do not cause infection in Humans, but influenza A (H7N9) and A (H5N1) virus strains tend to cause severe infections among people. This H5N1 has been linked with direct or indirect means by infected/dead poultry. It has seemed to cause infection in approximately all age group of individuals. Likewise in pandemic influenza, adolescents and young adults are disproportionately affected (Miotto et al., 2010; Fiebig et al., 2011; Zhang et al., 2011). Consumption of raw duck blood is suspected for some cases of Human H5N1 virus infection (Gambotto et al., 2008) and this mode of transmission is compatible with studies in mammals that intragastric inoculation of the

H5N1 virus can lead to systemic dissemination via the lymphatic's and venous route (Shinya et al., 2011). The H5N1 virus could be detected in preserved poultry in freezing condition and contaminated eggs, although transmission into the Humans through these food items routes has not been yet recorded (Harder et al., 2009). All pandemic or seasonal epidemic Human influenza viruses have a preferential binding for a2, 6 SA, which is predominant in the upper respiratory tract in Human and might be served as a precursor for efficient Human-to-Human transmission (Imai et al., 2012). Secondly, in addition to the host cell surface receptor requirement, the avian- and Human-adapted influenza viruses also have different importin-α isoform requirements (Gabriel et al., 2011). Finally, mutations that are responsible for Human adaptation seem to be unstable in H5N1 viruses (Miotto et al., 2010). To date, just the H5 and H7 subtypes have been ended up being HPAI infections, not all H5 and H7 infections are HPAI infections (Senne et al., 1996; Alexander 2007; Abdelwhab et al., 2011). In any case, LPAI H5 or H7 infections may move toward becoming HPAI infections because of changes that happen after the disease of poultry (Alexander, 2007).

H5N1 Outbreaks status

The epidemiology of avian influenza is complex. The virus continually evolves, the behavior of each new subtype (and strains within subtypes), the risks they present can vary in different countries. The epidemiology of AIV for the past thirteen years was characterized by two main global panzootics (Gao et al., 2018). The first panzootic wave started in 2004 peaked up in 2006, and progressively increase the virus activity which somewhat slows down in 2012. The Asian lineage shoes a remarkable continuation in its spread from countries in Asia and Africa in poultry and wild birds. The virus has become enzootic in Asia and Africa and continues to cause outbreaks in poultry and sporadic Human infections. Since 1997, an exceptionally pathogenic avian influenza virus of the H5N1 subtype had caused abundant episodes in poultry in Southeast Asia (Li et al., 2004; Chen et al., 2006; Smith et al., 2006) and have the range to more than 60 nations turned out to be more pathogenic in mammalian species (Chen et al., 2004; Maines et al., 2005). These flare-ups caused approximately fifty percentage of transmission from HPAI H5N1 to people. An occasionally refreshed timetable of H5N1 flare-ups can be found at as of February 2018, eight hundred sixty Human infections have been reported to WHO from sixteen countries and territories. All cases of H5N1 infection occurs due to its close contact with infected live/dead birds in H5N1 contaminated environment. The objective of this review is to provide a historical background of HPAI epidemiology from 2015 till present to provide context to the current situation and consider what might happen next (Table 1).

Disease transmission and its global distribution

The Northern Hemisphere winter season is mainly associated with an increased risk for AIV. New and reoccurring outbreaks of HPAI H5N8 and H5N6 in Asia, Europe, and the Middle East as observed in the 2017-2018 perspective. The zoonotic AIV that had been detected in China (H7N9 and H5N6) and some parts of Africa and Asia (H5N1), proved to cause the most significant public health risks. During the period January 2013 – January 2018, twelve different influenza A subtypes were reported by OIE 2018 (Adlhoch et al., 2018). Europe reported the highest virus diversity (seven subtypes), followed by Asia and the Americas (six subtypes each), Africa (three subtypes), and Oceania (one subtype) by OIE 2018 (Adlhoch et al., 2018). Subtypes H5N1, H5N2 and H5N8 were the most geographically widespread and commonly reported (Figure1).

Highly pathogenic avian influenza A (H5N1) virus had caused approximately 1,000 Human infections since the first case was reported in 1997 (overall case-fatality rate 54%) (Abubakar et al., 2016). The highest cumulative number of confirmed Human cases was reported in Egypt (Abdelwhab et al., 2016). This virus has been detected in poultry and wild birds in more than fifty countries worldwide and the virus is now epizootic in Bangladesh, India, China, Egypt, Indonesia and Vietnam.

Current Situation: Human H5N1 infection globally

According to reports received by the OIE, various influenza A (H5) subtypes continue to be detected and examined in birds of Africa, Europe and Asia. However, these Influenza A (H5N6) viruses are different from the A (H5N6) influenza viruses that have infected Humans in China (OIE, 2018). In the past five years, total of 1567 Human cases of H7N9 reported globally. Since March, 2014, there were 19 Human cases of avian influenza A (H5N6) reported globally and all occurred in Mainland, China (Hui et al., 2017). From 2011 to 2016, 10 to 145 confirmed Human cases of avian influenza A (H5N1) were reported to WHO annually. In 2017, there were three cases in Egypt and one case in Indonesia, WHO 2018 (Benitez, 2018). The latest case was reported on

September 27, 2017 by AIV report 2018 where avian influenza A (H7N4) virus was reported with one laboratory-confirmed Human case infection as reported to WHO. During January 26 to March 2, 2018, new Human infections with avian influenza A (H7N4), (H7N9) and (H9N2) viruses were reported (WHO, 2018). Thus, overall perspective to be depicted in Figure 2 for cumulative number of confirmed Human cases for avian influenza A (H5N1).

Reconnaissance in different quails

Quail (*Coturnix* spp.) had attracted and kept in attention because the internal genes of the H5N1 HPAI viruses detected in Hong Kong during 1997 showed quite a similarity to those found in an H9N2 virus in Quail (Guan et al., 1999). This finding may be suggested as a possible threat for this type of species in the evolution of the Hong Kong/97 genotype HPAI virus. Quail was also among the first avian species here it as reported that higher quantities of influenza virus could be excreted through the respiratory tracts as compared to faeces, as compared to earlier reports with AIV in aquatic avian species. H5N1 outbreak has been reported in many countries, mainly in Indonesia and Vietnam (Peiris et al., 2007). Practically, Quails are more prone to infection with H5N1 HPAI viruses (Perkins et al., 2001). Thus, they are no longer allowed to sell Quail in live and backyard poultry markets in Hong Kong, they can be the potential reservoir of other influenza viruses that could be linked with H5N1 viruses and its subtypes. From previous literature, quail also played a significant role in the supportive growth of swine influenza viruses (Makarova et al., 2003). Quails refer to 130 species of small, short-tailed game birds of the family Phasianidae (order Galliformes), resembling partridges but generally more modest and less robust. Common quail species include Bobwhite (*Colinus virginanus*), Chinese Painted (Button) Quail (*Coturnix, Excalifactoria chinensis*), Coturnix (*Japanese* or *Pharaoh*) Quail, Gambel's Quail (*Callipepla gambelli*), Mearns Quail (*Cytonyx montezuma*), Mountain Quail (*Oreortyx pictus*), Scaled Quail (*Callipepla squamata*), and California Valley Quail (*C. californica*) (Kennedy, et al., 1980). Old world migratory Quails (genus *Coturnix*) are classified by morphology and territorial range into their subspecies, range varies from one another as listed in IUCN Red list 2017. The European quail (E quail), also called common or wild quail, is a partial migrant whose breeding range extends from the Atlantic to Lake Baikal and from the Arctic Circle to the tropics (Del Hoyo et al., 1992). The Japanese quail (J quail) (*Coturnix c. japonica*), also called domestic quail, found in the wild in Asia (Barilani et al., 2005) best known for its domestic form in Europe, Asia, North America, and India where it is generally ranged in outdoor game farms for restocking and hunting purposes (Slota et al., 2011) as well as for meat and egg production (Mills et al., 1997).

Quails are susceptible to infection having several subtypes including both mammalian and AIVs (Figure 3). It served as one of the intermediate hosts, pushing up the generation of newly reassortment AIVs having remarkable potential to cause infection among Human (Thontiravong et al., 2012). The quail is a land-based bird commonly raised by Humans worldwide. Previous study showed that Quails can naturally be infected with various influenza subtypes as in avian, Human, swine origins such as H3, H4, H5, H6, H7, H9 and H10 subtypes, altogether with Human H1N1 and sine H3N2 influenza viruses (Liu et al., 2003; Nfon et al., 2011). Only viruses of the influenza virus A genus have been isolated from birds and termed AIVs, but viruses with all sixteen HA (H1-H16) and all nine NA (N1-N9). Quails are more susceptible than chickens to cause infection, and generation of recombinant H9 viruses by reverse genetics showed that changes in the HA gene are quite sufficient to initiate efficient replication mode and transmission in quail (Perez et al., 2003). Several finding suggested that quail supports an environment where in the adaptation of influenza viruses from ducks generated novel variants , which helps to cross the species barrier (Perez et al., 2003). Since the first reported case of avian influenza in J quail in Italy (1966–1968), influenza viruses of several subtypes have been isolated from quail in North America, Europe, and Asia through periodic surveillance and sporadic outbreaks (Guo et al., 2000, Suarez et al., 2000; Yee et al., 2011). J Quails may provide an optimal environment for the adaptation of wild bird AIVs, generating novel variants that can cross the species barrier to domestic poultry and Human beings. Infected birds showed neither clinical signs nor mortality. Virus isolation and real-time RT-PCR confirmed the presence of the H9N2 virus in cloacal swab samples collected at 35 days of age and the absence of other AIV subtypes, including H5 and H7. AIV was recovered from the internal contents of eggs, including a mixture of albumen and allantoic fluid, and from the oviduct of naturally infected J quail flocks in the southern part of Thailand.

Table 1. Latest report of highly pathogenic avian influenza virus's outbreaks in various global regions pandemic to poultry, migratory birds' species and Human's cases since 2015 to 2018 from worldwide

Global distribution	Regional distribution	Year	Culled birds/chickens (poultry to poultry transmission) (in numbers)	Avain influenza viruses subtypes	Human cases (poultry to Human transmission)
India perspective (Orissa, Telangana, Tamil Nadu regions, Maharashtra border districts, Delhi , Madhya Pradesh, Uttar Pradesh mainly Lucknow, on high alert since 2016 for H5N8/H5N1)	Bangalore	2018	942 cases	H5N8 / H5N1	No cases
	Maharashtra	2018	900 cases	H5N1/H7N9	150 cases show medical complications
	Ahmedabad	2017	1,50 cases	H5N1	No cases
	Telangana	2015	2 lakhs	H5 strains	No cases
	Manipur	2015	1,000	H5 strains	No cases
	Tripura	2016	8,500	H5 strains	No cases
Worldwide perspective (most of the countries Europe, Africa regions, Asia, Americas etc. for H5 and H7 serotypes)	China	2018	26 million	H7N9/H7N4	79 people died
	Netherlands	2018	230 cases	H5N6	1 people died
	Northwest provinces Madibeng and Macquarie Hills	2018	3 cases	H5 strain	No cases
	Japan	2017	310,000	H5N6	No cases
	South Korea	2017	12,300	H5 strains	No cases
	Japan's Gifu	2017	80,000	H5 strains	No cases
	Philippines	2017	470,000	H7N9	34 cases susceptible
	Bangladesh	2017	2,268	H5 strains	No cases
	Paris	2017	10,000	H5 strains	No cases
	South Africa	2017	260,000	H5N8	No cases
	Ireland	2016	550 cases	H5N8	No cases
	South Arabia	2018	38,000	H5N8	No cases
	North Holland	2018	230	H5N6	No cases
	Mexico	2018	539	H7N3	No cases

Transmission among different Quails and their comparative study: its viral shedding and bodily symptoms

Quails exhibited a high susceptibility to both HPAIV, as demonstrated by severe clinical signs and high mortality rates with its phenotypic characterization and its detailed information as depicted in (Table 2). Quails carry receptors of sialic acid with the potential and ability of binding of avian and Human influenza viruses hence, serving as an intermediate host for the zoonotic transmission of influenza viruses (Wan et al., 2006).The overall combination of gallinaceous species J. quail (Jeong et al., 2009; Saito et al., 2009). European quail (*Coturnix c. coturnix*) (E. quail) can be considered efficient shedders of HPAIV. With the earliest onset, the most rapid progression of the disease, and shortest in H5N1/HP-infected quail, it is apparent that this virus is more virulent for this species than the H7N1/HP. Quails have been proposed to be an intermediate host of influenza A virus. The pathogenicity, virus shedding, and transmission characteristics of pH1N1, swine H1N1, and avian H3N2 (dkH3N2) influenza viruses in Quails were examined in various studies E. quail may share with J. quail its potential as an intermediate host and reservoir of AIV. With the earliest onset, most rapid progression of the disease, and shortest MDT in H5N1/HP-infected quail, it is apparent that this virus is more virulent for this species than the H7N1/HP (Spickler et al., 2008). High level of pathogenicity were observed for both HPAIV corresponding with natural and experimental H5N1 HPAIV infections in chickens and other gallinaceous species, most importantly observed in J quail (Perkins et al., 2001; Jeong et al., 2009; Bertran et al., 2011). Previous studies reported minimal clinical signs or even sudden deaths without apparent symptoms in J quail (Perkins et al., 2001; Jeong, et al., 2009; Saito et al., 2009). Clinically neurological dysfunction was an evident sign in most of the HPAIV-infected quail of the present study. Certain gross findings indicative were not as extensive and obvious as for chickens (e.g., the presence of edematous, hemorrhagic, and necrotic cutaneous lesions), but affected tissues were known target organs for influenza A viruses in other gallinaceous species, including J quail (Perkins et al., 2001; Antarasena et al., 2006; Jeong et al., 2009; Saito et al., 2009; Bertran et al., 2011). Effective viral transmission from inoculated Quail

to naive contact birds was confirmed for the past studied viruses, even though their origin, avian hosts were as diverse as chicken, mallard, and great crested grebe. This finding suggests that adaptation may not be needed to allow AIV to replicate and transmit in E quail, confirming the substantial role that this species may play in AI epidemiology. As in previous work with H5N1 HPAIV in J Quail (Jeong et al., 2009), both HPAIV used in for study confirmed to be able to transmit among E quail. The HPAI H5N1 strains tested so far are all pathogenic in chickens (Hikono et al., 2013; Lee et al., 2013) and J Quails (Makarova et al., 2003; Isoda, et al., 2006; Jeong et al., 2009; Yamada et al., 2010). Chinese painted Quails are superior to that of chickens and J Quails due to their ease of handling, general care, hardiness, excellent reproductive performance, and less expensive maintenance (Tsudzuki, 1994). Chinese painted Quails is extensively used for vaccine studies with HPAI H5N1 viruses which requires enhanced biosafety level 3 conditions and provides an effective opportunity for the inclusion of the birds in appropriate numbers and groups for the experiments to achieve better scientifically remarkable and acceptable outcomes. Viral transmission among these birds like LPAIV probably occurs through the oral-oral route (Jeong et al., 2009; Saito et al., 2009; Sun et al., 2011; Bertran et al., 2013). Some HPAIV-infected gallinaceous birds, like E. quail may shed virus at high concentrations before the appearance of clinical signs and/or death. Therefore, spreading disease into the wild by releasing apparently healthy farm reared birds for hunting purposes could represent a substantial threat, which highlights the need for effective surveillance programs among these species. Projects undergoing discussed in table 3.

Table 2. Quails infected with highly pathogenic avian influenza viruses and its elaborative information

Quails more susceptible to highly pathogenic avian influenza viruses	**International Union for Conservation of Nature Red List by Birdlife international year 2016**	**Affected Place (year)**
Japanese quail *(Coturnix. coturnix japonica)* Temminck & Schlegel, 1849	Near threatened list	Hong Kong (1997)
European quail *(Coturnix. coturnix coturnix)* Linnaeus, 1758	Least concern list	Italy (1999), Spain (2009)
Bobwhite quail *(Colinus viriginainus)* Linnaeus, 1758	Near threatened list	Hong Kong (1997)
Chinese painted quail (Cotunix chinensis/Synoicus chinensis) Linnaeus, 1766	Least concern list	London (2006)

The references: Bertran K et al., 2013; Sarkadi et al., 2013; Perkins and Swayne, 2001

Table 3. Quail conservation programs undergoing worldwide

Name of the program for *Q*uail species	**The aim of the project**	**Country**	**Year**	**Land area under usage (acre)**	***Quail variety***	***Q*uail survey**
Unique restoration project by Denise Attaway, Forestry and Life Sciences; Public Service and Agriculture, 2018	Habitat restoration	New Jersey	2018	40,000	Northern Bob white *Quail*	320 Bob white (target 500 birds)
			Till 2015	16,000		117
Quail forever (commonly known as One stop shop)	Quail conservation	Texas	2018	17 million	United states naïve *Quail species*	13,000 habitat projects across the nation

The reference: Ammoland Inc. 2018

Virology and virulence factors

Gross observation and comparative study of the phylogenetic relationship of different types of AIV strains. The morphology of influenza A (H5N1) is essentially that of an orthomyxo virus as it's far a subtype of the kind A influenza virus. The typical virion is enveloped, round (100 nm), with a nucleocapsid of helical symmetry surrounding a minus sense, unmarried stranded eight segmented RNA. The envelope is internally covered by way of a matrix protein (M) and externally with glycoprotein peplomers-rod formed HA which might be homotrimers of sophistication I membrane glycoproteins and mushroom-shaped NA molecules which might be tetramers of a class II membrane protein. The H5N1 viruses acquired enhancedbird-to-Human transmissibility by (1) altering amino acids in HA that enable

bindingaffinity to Human-type receptors, (2) loss of the glycosylation site and 130 loops in the HA protein and (3) mutation of E627K in the PB2 protein to enhance viral replication in mammalian hosts (Kim, 2018). The genetic analysis of the virus HA suggested that the virus prefer to bind α (2–3)–linked sialic acids present in avian species. Adult Humans have a preponderance of α (2- 6)–linked sialic acids located in their upper respiratory tract, and H5N1 virus disease. Thus, the virus gains access to the alveolar epithelium where α (2-3)–linked sialic acids are present. It has been reported that young children differ in this respect and have a preponderance of α (2-3)–linked sialic acids in their upper respiratory tract (Short, et al., 2015).Vaccination in Human is still in the testing phase. Human studies have shown that antibodies against HA and NA could be able to elicit by vero cell and insect cell-derived vaccines (Ehrlich et al., 2008; Khurana et al., 2011). Phylogenetically informative amino acid positions (PIPs) were identified in influenza A NA of subtypes N1 and N2. NA evolves in a lineage-specific way as the virus adapts to a new host or changes to evade the host's immune system. Thus, deep study of viral genetic constituents may come out as a positive outcome for the present day perspective, if occurs due to the environment or genetic mutation as described in Figure 4 and 5. The surface proteins of influenza viruses, NA, plays anessentialrole in virulence, host specificity, and the Human immune response (Thomason, et al., 2012). Recent observations suggest that the potential role of quail (*Coturnix coturnix*) as intermediate hosts in the interspecies transmission of influenza viruses has been underestimated (Liu et al., 2003) and reported the isolation of a Human influenza A virus from the trachea of the quail.

Modes and detection

Several epidemiologic researchers have evaluated the danger of transmission of HPAI from poultry to people. These studies have identified several substantial chance factors that may be related to infection along with near direct touch with a rooster and oblique transmission through environmental infection. Direct routes of bird-to-Human infection of H5N1 can also encompass touch with aerosolized virus, infected blood or physical fluids via meals, education practices (e.g., slaughtering, boiling, defeathering, reducing meat, cleansing meat, eliminating and/or cleansing inner organs of hen), consuming uncooked products, or through the care of birds (both commercially or regionally). H5N1 transmission is progressively increasing day by day although, recent studies have cautioned an association between exposure to the contaminated surroundings (e.g., water, cleaning rooster cages or their specific areas, the usage of poultry faeces for fertilizer (Indriani et al., 2010, Gutiérrez et al., 2012) and disease through either ingestion or conjunctival or intranasal vaccination of defiled water and soil. Poultry markets have additionally been appeared to be a potential wellspring of H5N1 flow in poultry and disease source to people (Mounts, et al., 1999, Wang, et al., 2006, Abdelwhab, et al., 2010, Indriani, et al., 2010, Negovetich, et al., 2011, Samaan, et al., 2011). Because birds are known to shed high concentrations of the virus into water sources, transmission from poultry to Humans through contaminated water is also possible.

H5N1 RNA can be detected in the spleen, and can be cultured in the cerebrospinal fluid of infected Humans (De Jong et al., 2005). Currently, antigen detection by rapid immune chromatographic assays or direct immunofluorescence and nucleic acid detection by RT-PCR provides a quick diagnosis, which guides immediate management. In contrast, viral culture and serology allow for retrospective diagnosis, which is essential for epidemiological studies. Respiratory tract specimens are the best samples for detecting the virus, although the virus can also be found in blood or rectal swabs (De Jong et al., 2006). RT-PCR, HA restraint with horse red platelets as a corroborative test and DNA sequencing can prove to be helpful for detection purposes. Such testing ends up imperative when the poor inspecting system, poor example quality, or different issues discount segregation of the infection or block the utilization of PCR-based tests. The sequences of the primer sets defined in advance for PCR-primarily based detection of deadly disease may be suitable for the detection of virus traces presently circulating in Human beings (Claas et al., 1992; Claas et al., 1993; Cherian et al., 1994; Atmar et al., 1996) but display considerable numbers of mismatches when they are compared with the sequences of animal influenza A viruses. Many approaches still to come on the right path as an introduction from the US Centers for Disease Control and Prevention demonstrating many awareness programs in demonstrative testing, including lab affirmation of H5N1 contaminations given serological tests.

Bodily symptomatic and diagnosis

In contrast to Human seasonal influenza viruses, H5N1 viruses are more likely to cause severe pneumonia. In addition to pulmonary disease, H5N1 virus infection also leads to extra pulmonary manifestations more often

than infections caused by pandemic influenza viruses (To et al., 2010). Elevated creatine kinase is also common, but true rhabdomyolysis has not been described. The higher virulence of the H5N1 virus, which leads to a high pulmonary and extrapulmonary viral load, together with its intrinsic pro-inflammatory property, often causes a cytokine peak in these patients. Other common laboratory abnormalities included leucopenia, lymphopenia, thrombocytopenia and impaired coagulation profiles (Yuen et al., 1998). Serial specimens of CSF, nasopharyngeal aspirate (NPA), rectal swab, stool, urine, plasma, and serum were tested by real-time quantitative RT-PCR (qPCR) targeting M gene for influenza A virus and the HA gene for the H5N1 virus. Some case reports of H5N1 infections reported for manifestations of central nervous system (CNS) disease (Mak et al., 2017). Previous studies have indicated that chemokine levels were higher in fatal rather than nonfatal patients with H5N1 disease (De Jong et al., 2006). This amino acid substitution has been shown to increase the replication and pathogenicity of avian H5N1, H9N2, and H7N9 viruses in mammals in a manner analogous to previously reported mammalian adaptation signatures E627K and D701N (Mok et al., 2014).

Treatment and preventive strategy

Several antivirals in development have targeted important parts of the viral life cycle. Nucleozin, a NP inhibitor, has potent *in vitro* and *in vivo* activity against the H5N1 virus in a mouse model (Kao et al., 2010). The neuraminidase inhibitors oseltamivir or zanamivir are the mainstays of treatment for H5N1 infection (Table 4). Zanamivir is usually administrated by oral inward breath. Intravenous zanamivir, which was effectively utilized for Human during the 2009 H1N1 pandemic, is compelling against H5N1 infection disease in a macaque model (Stittelaar et al., 2008). Oseltamivir used as prophylaxis and orally controlled to people >1 yr. of age up to 10 days after a definitive exposure as reported (75 mg/d for grown-ups and 35 mg/d for youngsters). People are showing symptoms and manifestations of influenza, oropharyngeal swab examples were gathered and inspected at the national Influenza focus by using real-time RT-PCR (Farah et al., 2018). Determination of new immunization against infections (Farah, et al., 2018). Selection of new candidate vaccine viruses (CVVs) for zoonotic influenza causing pandemic was made during a recent WHO consultation. Various WHO authorities in collaboration with other centers for the overall epidemiology, its regulation and control situated all over the world (Figure 6). Currently, use of live attenuated influenza vaccines in poultry is not recommended by the OIE because of the ability danger of reassortment or transformations for producing AIV. Undoubtedly, the best immunization program to be brought upon, the segment infection should be observed for antigenic changes, and the antibody should be analyzed against new versions or at the very least the immunization should be re-assessed each 2– 3 years against currently circulating field viruses (Swayne et al., 2014). Nowadays, H9N2 detaches from quail have demonstrated antigenic buoy (Alderton 1992; Kandeil et al., 2017).

The antigenic relatedness of various sub-lineages needs further examination to select suitable vaccine strains. It can also be beneficial to reduce inter-governorate inter-regional movements associated with poultry trade through the promotion of regional trade to prevent the spread of AIV (Elmasry et al., 2017). The Food and Agricultural Organization of the United Nations has also recommended implementing a policy of not exchanging or carryover of animals (i.e., housing the same animals in the marketplace for multiple days), which improves live-market strategy to reduce AIV exchange.

Table 4. Collectively data set of influenza viruses for detection, diagnosis and treatment.

Subtypes combination detected	Antiviral drugs		Lab detection methods	Surveillance authority
Haemagglutinin (17 subtypes) and neuraminidase (10 subtypes) surface proteins.	Adamantanes, amantadine, rimantadine (currently not recommended for use due to widespread resistance)	Neuraminidase inhibitors, oseltamivir and zanamivir, developed in the 1990s, are effective against both influenza A and B viruses, and are widely available.	Genotypic assays and phenotypic assays	World Health Organisation Global Influenza Surveillance and Response System, World Health Organisation Collaborating Centres and some national influenza centres, World Health Organisation Expert Working Group on Surveillance of Influenza Antiviral Susceptibility

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CONCLUSION

In people, plenty latest studies have targeted at the factors that are responsible for the pathogenicity and transmissibility of the H5N1 virus. A few lines of confirmation recommend vital parts for the polymerase qualities. However, no single quality has yet been succeeded nor can the distinctive age profile of this disease be adequately explained at present. Notwithstanding, data about these infections and their related study of disease transmission in the locale is rare. Due to the lack of resources, early detection is one of the major problems in the developing countries. In affluent countries, detection is generally delayed due to the lack of awareness. Analysis of the viral genome with its virulence affecting pathway allows the scientific community to identify virulence determinants but proved to be little impact on a clinical level. Despite antiviral discovery and treatment procedures, many patients are still struggling to cope up with the viral disease and its associated symptoms. Since the introduction of neuraminidase inhibitors for more than 10 years back, no new antiviral that is active and capable of resisting outbreaks against the influenza virus have been yet approved. Many preventive strategies like proper vaccinations are effective in limiting H5N1 virus transmission in poultry but can prove more beneficially if limiting cross-species spread into poultry farms and the lack of cross-protection between different clades or subclades. Despite aggressive and triggered control measures in this particular area, sporadic Human H5N1 infections still occur, highlighting the need for high vigilance, especially when encountering patients who have poultry contact or have visited a poultry market. Despite worldwide surveillance, various schemes launched by the government regularly, periodically launching reported perspective timely into different avian linked sites and aggressive strategies to eliminate the H5N1 virus instead this virus continues to cause fatal outbreaks in both the avian and human population and causing havoc situation all over the world so on and so forth. The viruses can be evolved, generating many subclades with potentially enhanced virulence factors and remarkable transmissibility. Productive epidemiological observation frameworks are required additionally to permit the opportune distinguishing proof of new these infections presented in the Indian locale. It is essential to amplify local endeavours for the early discovery of AIVs in both nearby and transitory untamed life and to keep up biosafety and biocontainment boundaries to counteract disease in poultry.

DECLARATIONS

Author's contribution

Dr Vijay Laxmi Saxena has provided the conception and framework designing of the research article. Roshani Gupta and Khushboo Arya have involved in the data collection and analysis part. All the authors have contributed to drafting and critical revision of the article.

Competing interests

The authors have no conflicts to declare.

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Antibiotics Resistance in Broiler Chicken from the Farm to the Table in Eastern Algeria

Amine Berghiche[1,3*], Tarek Khenenou[1,2] and Ibtissem Labiad[3]

[1]*Department of Veterinary Science, Institute of Agronomy and Veterinary Science, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria*
[2]*Laboratory of Animal Production, Biotechnology and Health, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria*
[3]*Laboratory of Science and Technique of Living, Institute of Agronomic and Veterinarian Sciences, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria*
*Corresponding author's Email: amine_berghiche@yahoo.com

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ABSTRACT

A survey was carried out to collect information on the place of chicken meat in the feed ration of families. It aims at assessing the occurrence of diseases, the method of their diagnosis, the commonly used antibiotics in poultry farms and their impact on the health of humans in the North-Eastern region of Algeria. The survey was based on a questionnaire that was sent to 102 families, 50 poultry farmers and 30 veterinary practitioners in the poultry sector in the region. Our investigation has revealed that the Algerian families' consumption of chicken meat is the highest (85,3 %) compared with the other types of meats. As to the surveyed poultry farmers, the investigation has shown that most of them do not apply the residue disposal waiting times (70%). Concerning the surveyed veterinary practitioners, the investigation has, on the one hand, revealed that the cases of failure of antibiotic therapy are very common (96%), they primarily are due to the development of antibioresistance. It has, on the other hand, shown that veterinarians have become only drug distributors. These investigations have shown that there is a great lack of health monitoring, and a lack of quality of white meat. It has also been noted that there is a massive use of antibiotics and a dominance of anarchic use of veterinary drugs.

Key words: Antibiotics resistance, Consumers, Inquire, Poultry farmers, Veterinary surgeons

INTRODUCTION

During the last 30 years, sub therapeutic levels of antibacterial drugs have been fed extensively in every major livestock and poultry producing country (National Research Council, 1980). The poultry industry uses antibiotics to improve meat production by increasing feed conversion, promoting growth rates and preventing diseases. Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth (Berghiche et al., 2018; Barceló, 2007; Engberg et al., 2000; Kantati, 2011; KhodambashiEmami, 2012; Chattopadhyay, 2014) and protect the health of birds by modifying the immune status of broiler chickens (Lee et al., 2012).

In addition to bio-resistance, antibiotics abuse has resulted in drug residues in animal products (Gonzalez et al., 2017). Several antibiotics such as penicillin, tetracycline, macrolide, aminoglycoside and amphenicol have been detected in foods (Diarra and Malouin, 2014). However, the widespread, and even abusive use of some antibiotics, as curative, preventive or as food additives has led to the development of allergic reactions (Nisha, 2008) and to an increase in nosocomial risk and possible significant increase in the isk of contracting certain cancers (Dobson, 2008) and even to the development of populations of antibiotic-resistant microbes (Endtz and Ruijs, 1991; Allen et al., 1992; Zhang et al., 2003; Khenenou et al., 2011; Khenenou et al., 2012).

The aim of the present study is to highlight the place that chicken meat has in the feed ration of families and its effects on the quality of meat and on the health of humans.

MATERIALS AND METHODS

Ethical approval

The experiment was carried out according to the national regulations on animal welfare and institutional animal ethical committee.

Experimental design

The study was carried out in North-Eastern Algeria through the period from January 2017 to June 2018. The

survey is based on a questionnaire sent randomly to 102 families, 50 poultry farmers and 30 veterinary practitioners in the poultry sector in the region. Each one of the 102 families provided all personal references. Veterinary practitioners and farmers provided three professional references (surname and first name, address, Veterinarian Approval Number, authorization of poultry farming).

All the questionnaires were signed by the surveyed candidates. Questions have been made to collect information on the place of the chicken meat in the feed ration of the families, to assess: the occurrence of diseases; the method of their diagnosis, the commonly used antibiotics in poultry farms and their impact on the health of humans and the therapeutic approaches followed to understand the impact of self-medication phenomena in the region.

Statistical analysis

The collected data are logged and processed using the program (Microsoft Office Excel, 2007) to perform the description and evaluation.

RESULTS AND DISCUSSION

In order to have a clear idea of the various aspects that this study deals with, we have tried to take advantage of all the information that the answered questionnaires provided.

Knowledge of the poultry farmers of the nature of all the drugs used

72% of poultry farmers knew the nature of all drugs used during the rearing period and their dosages (Table 1). Present results are close to the findings of Elmanama et al. (2016) who reported that 87.9% of poultry farmers knew the nature of the drugs and the risks related to an unreasonable use of antibiotics on the health of humans.

Routes of antibiotics administration

The oral route is the most widely used route of administration (98%, Table 2). This is the easiest method being practiced by farmers and offers the possibility of distributing large volumes to several birds at once. This result is close to those obtained by Chevance et al. (2012); Khalen-wouembe et al. (2013); Khenenou et al. (2014); Sinaly (2014); Berghiche et al. (2017) and Khenenou et al. (2017) who found that the oral administration was the commonly used route in poultry farms.

Waiting period

Concerning the waiting time between the administration of the drugs and the slaughtering, it has been shown that 70% of the poultry farmers apply the waiting period; 64% of the farmers stop giving the drugs before the commercialization (Table 3). This result is similar to the reports of Bada-alambedji et al. (2004) who found that 70.7% of farmers applied the waiting period. Another survey conducted by Nkaya (2004) in Dakar revealed that 84.62% of farmers applied the waiting period.

Impact of the antibiotics on the health of humans

Our study has revealed that 72% of the surveyed vets have confirmed that there is a risk for the health of humans related to the unreasonable use of drugs (antibiotics) (Table 4). According to our results, the majority of farmers are aware of the risks of unreasable use of antibiotics.

Table 1. Knowledge of poultry farmers of used veterinary drugs, eastern Algerian (January 2017 to June 2018)

Knowledge of the poultry farmers of the nature of all the drugs used	Numbers	Percentage
Yes	36	72%
No	14	28%
Total	50	100%

Table 2. Antibiotic administration routes

Routes of administration of antibiotics	Numbers	Percentage
Drinking water	49	98%
Injection	3	6%
Food	6	12%
Total	50	100%

Table 3. Application of the waiting periods of drug administration by the poultry farmers, eastern Algeria (January 2017 to June 2018)

Waiting period	Numbers	Percentage
Yes	35	70%
Not	9	18%
Sometimes	6	12%
Total	50	100%

Table 4. Poultry farmers' awareness of the risks that antibiotics have on the quality of chicken meat and on the health of humans, eastern Algeria (January to June, 2017)

Farmers' awareness of the risks of antibiotics for the health of humans	Numbers	%
Yes	36	72%
No	14	28%
Total	50	100%

The frequency of self-medication in the poultry farming Self-medication in the poultry industry

The study has showed that more than 56% of the surveyed vets have declared that self-medication in the poultry farms happens quite often (Figure 1). The results are very close to those found by Sinaly, who found that 79% of farmers practiced self-medication. Hence, they are superior to those of Khalen-Wouembe (2013) who found that 33.64% of farmers, in the western region of Cameroon, did practice self-medication.

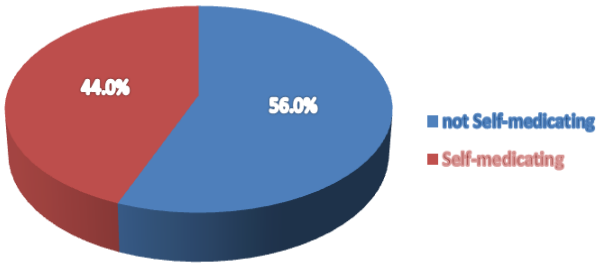

Figure 1. The frequency of self-medication in the poultry farming, eastern Algeria (January 2017 to June 2018)

The problem of antibiotic resistance

Nearly all veterinary surveys have shown that cases of treatment failure are common in the field and most antibiotic molecules are involved (Figure 2). These failures, according to the surveyed veterinarians, are mainly due to the development of antimicrobial resistance (96%). It is important to emphasize that any use of antibiotics eventually leads to the development of resistant microbial strains. The risk of developing resistance is due to the frequent and widespread use of a large proportion of antibiotics in henhouse (Chevalier, 2012).

Low-level antibiotic feeding has resulted in bacterial resistance (Linton, 1977 a; Linton, 1977 b; Braude et al., 1978; Richmond et al., 1980). Large differences have been found in drug resistance of E. coli between animals fed and those not fed antibiotics. The resistance to antibiotics is transmissible. In many instances the resistance is due to the presence of R-plasmids. These R-plasmids are able to mediate their own conjugal transfer in addition to specifying resistance to certain antibiotics ((Linton, 1977 a; Linton, 1977 b; Braude et al., 1978; Richmond et al., 1980).

However, Linton (1977a and 1977b) reported that the spread of the phenomenon of antibiotic resistance is due to the total absence of health monitoring; persistence of drug resistance has been related to the usage pattern of antibiotics.

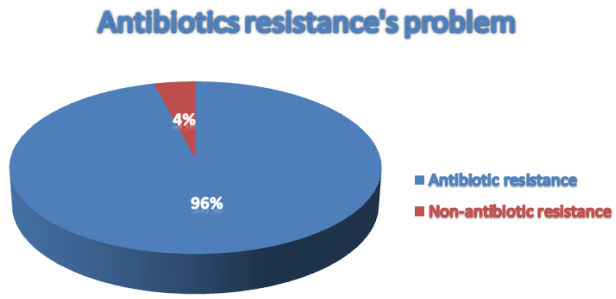

Figure 2. Antibiotics resistance's problem in broiler chicken, eastern Algeria (January 2017 to June 2018)

Frequency of consumption of chicken meat

According to the survey, we have found that chicken meat is highly consumed (85, 3%) compared to other meats mainly because of its low price and good taste. Our results are higher than those of Ramdane (2015) who found that 67% of families consumed chicken meat more than sheep and beef meats.

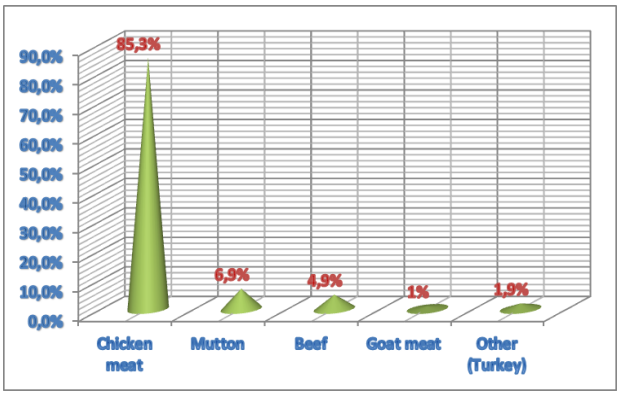

Figure 3. Types of meat consumed in east of Algeria (January 2017 to June 2018)

Place of purchase and type of chicken consumed

Butchers are the main place of purchase because of the hygienic conditions and the medical monitoring of this meat compared to other places of purchase. Modern farmed chicken is more consumed because it is available as carcass in butcheries, whereas farmed chicken requires preparation (sacrifice, plucking and evisceration). It is for these reasons that the public health community and FDA have been proposing to limit use of antibiotics on livestock for more than three decades (see list below). Consumers Union believes that as a prudent measure, we should drastically reduce use of antibiotics on food

animals, and eliminate use altogether for growth promotion or disease prevention in healthy animals (National research council committee, 1980).

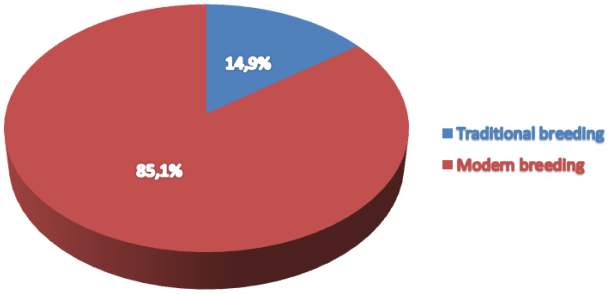

Figure 4. Type of chicken (Hubbard F15) consumed by family in eastern Algeria (January 2017 to June 2018)

CONCLUSION

On the basis of the above results, it can be argued that the Algerian consumer does not have a culture of choice and quality of the products to be consumed, and ignores the importance of the commodity and its origin, whereas he considers the price as the only reference.

Our investigation showed that there is a great lack of medical monitoring and poor traceability of the quality of broiler meat from the farm to the Algerian consumer compared to their counterparts in Tunisia and Morocco; as well as the developed countries, which have put an end to this problem since ten years

DECLARATIONS

Competing Interests

The authors have no competing interests to declare.

Consent to publish

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

Author`s contributions

Berghiche, Khenenou and Labiad were involved in the collection of data, statistical analysis and drafting of the manuscript. Khenenou and Berghiche read and approved the final manuscript.

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Biochemical Alterations in Hypervitaminosis D_3 in Broiler Chicks Concomitantly Challenged with Endotoxin

Rahul Kumar[1]*, Rajinder Singh Brar[2], Harmanjit Singh Banga[2] and Sandip Sodhi[2]

[1]*Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science & AH, DUVASU, Mathura-281001, India*
[2] *Professor Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India*
*Corresponding author's Email: rahulpoultrypatho@gmail.com

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ABSTRACT

Vitamin D_3 is ten times more biologically active than vitamin D_2, over supplementation of vitamin D_3 causes hypercalcemia with deposition of calcium and phosphate as crystals in the visceral organs. Birds are considered more resistant to endotoxin and information on inflammation and homeostasis in birds supplemented with higher dose of vitamin D_3 when suffer endotoxic shock is lacking. The present study was conducted to compare the effect on hemoglobin concentration and biochemical parameters of broiler chicks by administering toxic dose of vitamin D_3 for 21 days concomitantly challenged with endotoxin. The chicks were randomly divided into four groups viz. A, B, C and D. Hemoglobin concentrations of control groups (A and B) and treatment groups (C and D) did not differ significantly ($P<0.05$). Hypercalcemia and hyperphosphatemia was observed in both treatment groups in comparison to the control group. No significant ($P<0.05$) change was observed in the concentrations of total protein and albumin and in the activity of plasma Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase on day 28 of control (A and B) and treatment (C and D) groups. Vitamin D_3 supplementation causes immunomodulation; hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

Key words: Broiler chicks**,** Hypercalcaemia, Hypervitaminosis D_3, Hyperphosphatemia,

INTRODUCTION

Vitamin D3 is a fat-soluble vitamin, which originates from pro-vitamins ergosterol and 7-dehydrocholesterol based on activity of sun radiation. Vitamin D is added to diets in its crystal form as cholecalciferol (vitamin D_3) which is converted to 25-hydroxycholecalciferol (calcidiol, 25-OH D_3) in the liver and is further converted to the active metabolite 1,25-dihydroxycholecalciferol [1,25-(OH)2 D_3] in the kidneys (Beasley, 1999; Adams, 2010; Kelly et al., 2016). 1,25-dihydroxycholecalciferol (calcitriol) supports calcium and phosphorus absorption in the intestine, affects bone calcification and co-participates in calcium and phosphorus metabolism in the organism (Price et al., 2001; Cheng et al., 2016). In poultry, vitamin D_3 is ten times more biologically activity than vitamin D_2 (Soares et al., 1995; Fritts and Waldroup, 2003; Wideman et al., 2015). Vitamin D_3 is supplemented in the diets of poultry and because the optimum levels of dietary vitamin D_3 are rarely known, there is always a risk of over-supplementation (Nain et al., 2007), which causes hypercalcemiaemia and promotes deposition of calcium and phosphate as crystals in the kidneys, heart and major blood vessels (Cheng et al., 2016; Armstrong et al., 2018).

Lipopolysaccharides (LPS) are cell wall components of Gram-negative bacteria, which cause release of cytokines that regulate different metabolic responses and cause fever, inflammation and cachexia (Abbas et al., 1997). It is considered that birds might be relatively more resistant to endotoxins than mammals (Roeder et al., 1989) and Vitamin D_3 supplementation provide immunomodulation (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016; Kelly et al., 2016).

There is a paucity of information on the effects of LPS on cytokines and the acute phase response and their

relationship with inflammation and homeostasis in birds supplemented with higher dose of vitamin D_3, hence, the study is framed to find out the effect of higher dose of vitamin D_3 concomitantly challenged with endotoxin on biochemical parameters of broiler birds.

MATERIALS AND METHODS

The broiler chicks of strain IBL-80 procured from the hatchery, department of animal genetics and breeding (AGB), college of veterinary science, GADVASU, Ludhiana, India were kept for 7 days to acclimatize in laboratory conditions prior to start of sampling protocols. On day 7, the chicks were randomly divided in 2 broad groups (n=16 chicks) viz. treatment group and control group. Treatment group was administered with vitamin D_3 @ 2.5mg/kg body weight (BW) in groundnut oil daily by oral route. On day 28, 8 chicks from the control group were instilled with Normal saline solution (NSS) @ 0.5ml/chick (group A) and other 8 chicks were challenged with Lipopolysaccharide (LPS) @ 0.5ml/chick by intranasal route (group B) and then sacrificed after 12 hours of challenge with endotoxin. Similarly, 8 chicks from the treatment group were challenged with NSS @ 0.5 ml/chick (group C) and other 8 chicks were challenged with LPS @ 0.5 ml/chick by intranasal route (group D) on day 28 of the study. Blood samples (n=32) from wing vein for hemogram from all the broiler chicks of each group were collected 12 hours post endotoxin challenge in heparinized vials following the standard protocols of institutional animal ethical committee.

Plasma was separated and stored in aliquots at -20^{o}C till further use for analysis of various analytes viz. Plasma calcium concentration, plasma albumin etc. and processing for biochemical parameters (mean level of plasma calcium, phosphorous, albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were carried out at the end of experiment (day 28). The hemoglobin was estimated by using standard protocol (Benjamin, 1985). The plasma biochemical parameters were estimated using auto analyzer (BIOTRAN BTR-830) and diagnostic reagent kits supplied by Siemens India Limited, Gujarat, India using manufacturer protocols.

Statistical analysis

The statistical analysis was performed using One-Way Analysis of Variance (ANOVA) and all data were analyzed by using SPSS software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.). The results were presented as mean ± SEM (standard error of mean). Results were considered significant if $P<0.05$.

Ethical approval

The study was conducted without affecting the birds' general wellbeing. Approval was taken from concern authority.

RESULTS

The effect of oral administration of vitamin D_3 and intranasal LPS on biochemical parameters in chicks on day 28 has been summarized and presented in table 1. The level of hemoglobin in treated chicks did not differ significantly ($P<0.05$) from control chicks. The mean plasma calcium and phosphorous concentration in chicks of treatment groups (groups C and D) were significantly ($P<0.05$) higher than chicks in control groups (A and B). The mean plasma total protein, albumin concentration, ALT, AST and ALP in chicks of treatment groups did not differ significantly ($P<0.05$) from control groups.

Table1. Effect of oral administration of vitamin D_3 (2.5mgkg^{-1}day^{-1}) and intranasal lipopolysaccharide (0.5 mg bird^{-1}) on biochemical parameters in broiler chicks on day 28 (Mean±SE)

Parameter	Control groups		Treatment groups	
	Group A (n=8) (Mean±SE)	Group B (n=8) (Mean±SE)	Group C (n=8) (Mean±SE)	Group D (n=8) (Mean±SE)
Hb (g/dL)	12.19±0.33	12.06±0.22	12.40±0.50	12.00±0.20
Calcium (mg/dL)	10.05±0.56^a	9.48±0.31^a	14.41±0.30^b	14.17±0.58^b
Phosphorous (mg/dL)	4.97±0.28^a	4.65±0.16^a	6.03±0.17^b	5.82±0.23^b
Total protein (g/dL)	5.72±0.12	5.56±0.21	5.28±0.18	5.62±0.19
Albumin (g/dL)	3.38±0.18	3.38±0.18	3.38±0.18	3.25±0.16
ALT (U/L)	12.23±0.96	12.55±1.15	10.92±1.56	10.27±1.06
AST (U/L)	226.00±8.44	211.75±10.40	222.62±8.50	240.88±11.19
ALP (U/L)	1278.12±79.18	1216.38±99.13	1151.50±86.07	1101.75±76.32

Group A: control + Normal Saline Solution; Group B: control + LPS; Group C: Vitamin D_3 @ 2.5mg/kg BW + NSS; Group D: Vitamin D_3 @ 2.5mg/kg Body Weight + LipoPolySsaccharide; $^{a, b}$ values within a row lacking a common superscript differ significantly at $P<0.05$.; Hb; haemoglobin, ALT; Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase

DISCUSSION

Administration of vitamin D_3 @ 2.5mg/kg BW for 21 days did not influence the mean hemoglobin concentration. The chicks of the group D which were administered vitamin D_3 @ 2.5mg/kg BW orally daily and challenged with intranasal LPS 12 hours before sacrifice caused no alteration in hemoglobin concentration as compared to control chicks that only challenged with intranasal LPS 12 hours before sacrifice. These results are in agreement with Borissov and Andonova (2000) and Kumar and Mallik (2001) who reported no change in hemoglobin concentration in piglets and calves respectively on endotoxin exposure. However, the results of the present study are in contrast with the findings of Roberson et al. (2000) who conducted vitamin D_3 toxicity in lambs and reported increased hemoglobin and hematocrit values.

Bahman et al. (2011) reported normal plasma/serum calcium in the range of 9-10.5 mg/dL in broiler chicks. In the present study treated groups showed hypercalcemia. The toxic effects of vitamin D_3 are primarily related to the role of vitamin D_3 in the regulation of plasma calcium (Davies and Adams 1978; Reichel and Norman, 1989). The active metabolites of cholecalciferol have been reported to increase the blood calcium level by increased resorption/mobilization of calcium from bone, increased absorption of calcium from intestine and decreased calcium excretion by kidney (Vieth, 1990; Lumeij, 1994; Norman, 1996; Pettifor et al., 1995). Taylor et al., (1968) reported that in chicks given the toxic level of vitamin D_3 there was an increase in plasma calcium, similar results were reported by authors in their studies (Mazumdar et al., 2017; Cheng et al., 2016) whereas no increase in serum calcium level was reported by Cavia et al. (2015) in guinea pigs. Armstrong et al. (2018) reported increased plasma calcium level in dogs prophylactically given calcitriol. Vitamin D_3 toxicity results to higher blood calcium level leading to renal and cardiac failure culminating to death (Beasley, 1999; Radostits et al., 2000; Price et al., 2001) in different species.

Phosphate flux through the gastrointestinal epithelium is enhanced by vitamin D_3. This results from a direct effect of 1,25-dihydroxycholecalciferol, this hormone's action on calcium absorption, the calcium in turn acting as a transport mediator for the phosphate. Vitamin D_3 also increases calcium and phosphate reabsorption by the epithelial cells of the renal tubules, thereby tending to decrease excretion of these substances in the urine (Guyton and Hall, 2001). Similar results were reported by Cavia et al, (2015) in guinea pigs, on the contrary Linda and Aaron (2016) reported decrease in phosphorous concentration as a result of vitamin D_3 supplementation in laying hen. The normal levels of plasma/serum phosphorous in the range of 4-6 mg/dL in apparently healthy broiler chicks (Bahman et al., 2011).

The normal plasma total protein and plasma albumin level in vitamin D_3 toxicity in present study was dissimilar with low total protein level in vitamin D_3 toxicity reported by Roberson et al. (2000) in lambs. The findings in the present study are in contrast with Beasley (1999) who reported clinical signs like proteinuria and hence decrease in plasma total protein concentration in vitamin D_3 toxicity. The non-significant ($P<0.05$) change in the plasma total protein and albumin values may be due to dehydration because of polyuria and hence no change in relative protein concentration of plasma. The pathological changes in the kidneys (mineralization) might have prevented the excretion of plasma proteins and albumin via urine hence, maintaining the concentration of these proteins in the plasma. Bosch et al. (1988) and Al-Dughaym (2004) reported decreased total protein and albumin concentration in endotoxin treated animals. However, in the present study plasma total protein and albumin concentration did not show any significant ($P<0.05$) alteration between the chicks of group B and D exposed to LPS.

The serum transaminases (ALT and AST) are liver specific enzymes and thus their values help in detection of hepatocellular injury (Tenant, 1997). In the present study there was no alteration in their values which might be attributed to normal histoarchitecture of liver of the treatment groups. However, significant ($P<0.05$) increases in serum transaminases have been reported in cross bred calves (Kumar and Mallik, 2001), in rabbits (Yajar et al., 2004) and in pigs (Borissov and Andonova, 2000) challenged with endotoxin. Moreover, it has been opined that damage to any particular organ cannot be cited as cause of increased level of serum transaminases (Kaneko et al., 2008).

There was no significant decrease ($P<0.05$) in the plasma ALP activity in treatment group C and group D. This may be due to decreased osteoblast cell activity and increased osteoclast cell activity in bones because of high dose of vitamin D_3. Acute doses of vitamin D_3 (>100 times than required level) can result in negative calcium balance because of bone resorption is accelerated as in *Solanum malacoxylon, Cestrum diurnum and Trisetum flavescens* toxicity (Kaneko et al., 2008). Cavia et al. (2015) reported increase in ALP activity in

hypervitaminosis D in guinea pigs. ALP activity of LPS challenged chicks (groups B and D) did not reveal any significant ($P<0.05$) difference from NSS challenged chicks (groups A and C). Similar results were reported was comparable to control group chicks (group A) in crossbred calves (Kumar and Mallik, 2001) and in rats (Bosch et al., 1988) reported similar findings in endotoxemia. This non-significant decrease ($P<0.05$) in ALP activity in treated groups (group C and D) might be due to mineralization causing damage to kidney.

There is no significant change in the hematological and biochemical parameters of broiler chick, this may be due to the immunomodulation provided by Vitamin D_3 fed to the broiler chicks. Different authors in their studies confirmed similar immunomodulatory effects of Vitamin D3 (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016).

CONCLUSION

The Vitamin D_3 is an immunomodulator; broiler diet supplemented with Vitamin D_3 causes immunomodulation in broiler birds, hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

DECLARATIONS

Consent to publish

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

Competing interests

The authors declare that they have no competing interests.

Author`s contribution

All authors have equally contributed in this work.

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Effect of Combination of Encapsulated Black Cincau Leaves (*Mesona Palustris* BL) and Probiotics on Production Performances, Yolk Cholesterol Content and Ammonia Level of Laying Hen

Muhammad Halim Natsir[1*], Osfar Sjofjan[1], Ilham Ardiansah[2], Siti Khairani[2] and Elliyana[2]

[1]*Lecturer of Department of Animal Feed and Nutrition Science, Faculty of Animal Science, Universitas Brawijaya, 65141, Malang, Indonesia*
[2]*Undergraduate student of Department of Animal Feed and Nutrition Science, Universitas Brawijaya, 65141, Malang, Indonesia*

*Corresponding author's Email: emhanatsir@ub.ac.id

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ABSTRACT

The purpose of this research was to determine addition of natural feed additives from combination of encapsulated black cincau leaves and probiotics on feed intake, feed conversion, hen day production (HDP), egg mass, income over feed cost (IOFC), egg weight, yolk cholesterol content and ammonia levels in excreta. One hundred ninety-two laying hens at 28 weeks were used in this experiment. Egg mass which used before this research was 64.63±2.97 g/ day with CV was 4.59%. The method which used was experimental of completely randomized design (CRD) with four treatments and six replications (eight-layers each). The treatments used were T0: basal feed; T1: basal feed + combination of encapsulated black cincau leaves and probiotics 0.5%; T2: basal feed + combination of encapsulated black cincau leaves and probiotics 1%; T3: basal feed + combination of encapsulated black cincau leaves and probiotics 1.5%. Data were analyzed by using analysis of variance, if any significant effect, it would be further tested by Duncan's Multiple Range Test. The result showed that no siginficant effect (P>0.05) on feed intake, feed conversion, HDP, egg mass, IOFC, egg weight and yolk cholesterol content, but any significant effect (P< 0.05) on ammonia level. This research concludes that using 1.5% of combination of encapsulated black cincau leaves and probiotics give better result than others.

Key words: Black cincau leaves, Egg quality, Encapsulated probiotic, Hen production, Laying hen

INTRODUCTION

The term of probiotic mean life microorganism which have beneficial effect for its host. Probiotics are classified by the US Food and Drug Administration as generally recognized as safe (GRAS) ingredients (Patil et al., 2015). Commonly syntetic Antibiotics Growth Promoter (AGP) was used as growth promoter in poultry. AGP was used by farmer to increase egg production. Otherwise, using uncontroled AGP produce chemical residue on laying hen and its eggs, which can be harmful for human health. AGP was under supervised by many association, and have been removed from many countries (Ratcliff, 2000).

Developing sustainable livestock in poultry sector in the world should be considered as a good solution. Nowdays, the researchers are not only to focus on the enhancement of productivity, but also on food healthy and safety (Sienny and Serli, 2010). Especially in the developing country of Indonesia, this tropical country has high temperature and humidity. Those conditions could trigger heat stress on egg of laying hen (Li et al., 2015). Heat stress is able to disturb the health and reducing feed intake, then followed with decreasing productivity. Feed in poultry farm have highest production cost (approximately 70-80%). That all of the reason, feed should be efficiently used. Feed efficiency can be improved by adding feed additive (Teguie et al., 2004).

Using natural feed additives is needed. Natural feed additive might use probiotics and phytobiotics from herbal plants. Probiotics is single culture or mixture of living consumable microbes for human and/or animals and it has beneficial effect for its host (Patil et al., 2015). Its mechanism process is done in order to preserve the natural microflora balance within the body. Several benefit of addition of probiotics as feed for chicken would be decreasing mortality and cholesterol, increasing

hemoglobin (Hb) concentration, Packed Cell Volume (PCV), villus height also inhibit *Eschericia coli* and *Enterococci* (Rahman et al., 2013; Vantsawa et al., 2017; Bitterncourt et al., 2011; Pourakbari et al., 2016; Song et al., 2014). Cholesterol is a fat component composed of triglycerides, free fatty acids, and phospholipids. Eggs contain protein, carbohydrates, water, vitamins, mineral and cholesterol content, in one egg reaches 230,7 mg/ 100 g (USDA, 2018).

Black cincau is underbrush plant with height between 30-60 cm and mostly grown in 150-1800 m above sea level. Its leaves contain bioactive compound such as antioxidants, antibacterial, antimutagenic, hepatoprotective, antihypertensive and antidiabetic properties. Those bioactive compounds supposedly can maintain gastrointestinal tract and improve small intestinal microflora, performances and egg quality of laying hen (Hung and Gow, 2002).

Combination of black cincau leaves and probiotic has potentially given positive effect. This combination is expected to improve the ability of digestive system and also to improve the digestive enzyme activities, so that it could absorb feed nutrients for its production. Combination of phytobiotics and probiotics were characterized as hygroscopic. High vulnerable potency to beat heat condition, should protected by encapsulation technology. Microparticles should be water-insoluble to maintain their structural integrity in the food matrix and in the upper part of the GI tract that was needed to preserve the matrix content, benefit of these compounds during processing and storing, also to preserve probiotics viability (Chavarri et al., 2012). Encapsulation technology can be used as an alternative oral delivery to maintain the viability of probiotics, and allow it in controlling the release of viable cells into the host gut (in small intestine) so that it can be benefits (Cook et al., 2014).

Encapsulation is the process of coating one or more material to protect sensitive materials, in this case is feed components. It can reduce feed degradation in small intestine. According to Natsir et al. (2013), polymer that often used was Arabic gum, skim milk and whey protein which are able to prevent oxidation in feed additive, preserve the bacteria to stay alive during storage and better emulsion.

Based on that brief description, a study concerning a combination of encapsulated phytobiotic from black cincau leaves and probiotic were observed on this research. This research was conducted to know about production performance (feed intake, Hen Day Production (HDP), egg mass, feed conversion, Income Over Feed Cost (IOFC), egg weight, ammonia level) and yolk cholesterol content of laying hen.

MATERIALS AND METHODS

Materials and diets

One hundred ninty two 28-week-old strain Lohmann brown were used tin this experiment. Pre-experiment showed that the HDP was 84.57%, egg mass average was 64.63±2.97 g and variation coefficient was 4.59%. Birds then were housed in 24 group cages sized 64 cm ×35 cm ×30 cm (eight birds each) at 25-32 ^{o}c. The cages were equipped with lighting for 16 hours, feeder, drinker, egg container and digital scale (5 kg capacity), in order to measure feed residue and egg weight every day.

Black cincau leaves were obtained from Ponorogo Regency, East Java province. Probiotics used in this study were *Lactobacillus* sp 5.4 ×10^{7} cfu/ g and *Bacillus* sp 2.4 ×10^{8} cfu/ g. Encapsulation process was made using 20 g arabic gum, 5 g whey and 0.06 g butylated hydroxy toluene, 100 g of green black cinacu leaves were extracted with ethanol 70% and then mixed with mixer at 2500-3000 rpm for 15 minutes. Furthermore, the extraction was dried in modified microwave at 60^{o}C for 20 minutes. Composition of basal feed and nutrients content in proximate analysis were showed in table 1.

Experimental design

This research using four treatments and six replications (eight birds each). Feed was given as restricted feeding, 120 g/ hen/ day and drink as ad libitum for five weeks (35 days). Feeding frequency was done once a day, in the morning. Experimental feeding method was done by mixing basal feed with encapsulated of black cincau leaves-probiotics. The treatments were used as follows: T0 = basal feed; T1 = basal feed + combination of encapsulated black cincau and probiotics 0.5%, T2 = basal feed + combination of encapsulated black cincau and probiotics 1%; T3 = basal feed + combination of encapsulated black cincau and probiotics 1.5%. Data were analyzed by completely random design of ANOVA. If there was significant ($P<0.05$) then it is tested using LSD test (Steel and Torrie., 1980).

Ethical approval

This research did not involve the introduction of any intervention on birds. The data collcetion was obtained with humanly handled, which according of animal care and welfare standard of Republic Indonesia.

Table 1. Composition and nutrients content of basal feed[1]

Feedstuffs ingredients	Percentage (%)
Soybean meal	19.42
MBM	7.96
CGM	0.97
Maize	48.56
Rice bran	14.57
Premix	1.75
Binder	0.19
Salt	0.19
O-lalat	0.005
Orgacid	0.05
Vitamin C	0.01
Grit	6.31
Total	100
Analyzed feed composition	**Content**
Dry matter (%)	88.89
Ash (%)	13.87
Crude Fiber (%)	4.17
Crude Protein (%)	19.90
Crude Fat (%)	4.59
Gross Energy (MJ/ kg)	16.58
Metabolizable Energy (MJ/ kg)	11.61

[1]Proximate assay analyzed by Nutrition and Animal Feed Laboratory, Animal Science Faculty, Universitas Brawijaya, MBM= meat bone meal, CGM= corn gluten meal, MJ/ kg= megajoule per kilogram

RESULT AND DISCUSSION

Effect of combination of encapsulated black cincau leaves (*Mesona palustris* BL) and probiotics on production performances and yolk cholesterol content of laying hen have observed. The result in this research showed in Table 2.

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on feed intake and feed conversion

The result has shown in table 2. There is no significant effect (P<0.05) of combination of encapsulated black cincau leaves (*Mesona palustris* BL) and probiotics on feed intake and feed conversion. Feed intake in this research (T0, T1, T2 and T3) were reported 117.62 g, 118.34 g, 116.68 g, 118.05 g, consecutively. This result has been supposed to refer to the restricted feeding management which given once in a day. Furthermore, encapsulated black cincau leaves and probiotics have not influence on palatability, which respectable to feed intake. Factor affected feed intake were body size, genetic trait (breed), temperature, cage condition, feeder, condition of drinking water, quality and quantity of feed also the existence of disease. Oyedeji et al. (2005) reported that feed intake was influenced by feedstuffs and feeding method, such as feeding pigments. Moreover, different average value for each treatment would probably due to environmental factor.

There was significant difference (P<0.05) on feed conversion (Table 2). This might be supposed feed intake and egg mass in this research were also showed no significant difference (P>0.05). Feed conversion were reported 2.26; 2.28; 2.21; 2.11, consecutively. Several factor that influencing feed conversion were feed physical form, body weight, feed nutritional content, nursery environment, strain and sex. Another factors, such as temperature condition, damage of feed, feed quality and different location of housing have been reported to affected on feed conversion (Kelebemang, 2005; Suganthi et al., 2011; May and Lot, 2000).

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on HDP, egg mass, IOFC and egg weight

The result of HDP, egg mass, IOFC and egg weight have been shown in table 2. There was no significant difference (P>0.05) on those variables. HDP was reported, there was no significant difference (P>0.05) in this research. HDP result of this study were 84.57%, 84.64%, 83.00%, 89.80%, consecutively. It is supposed

due no significant difference (P>0.05) on feed intake in this study. Egg shaping of laying hen also depend on feed, which have been determined by its protein, fat, and calcium content. HDP is always related with egg shaping- production, thus egg production affected by feed intake and environmental condition. Reported from Awoniyi (2003) that different stage of housing influenced HDP of laying hen.

There was no significant effect (P>0.05) on egg mass in this research. Egg mass (g/ hen/ day) was reported as follow 53.24, 52.79, 53.72, 57.27, consecutively. This might occur because the addition of encapsulated of black cincau leaves and probiotic have not significant effect (P>0.05) on HDP. Measuring of egg mass (g/ hen/ day) was HDP × average egg's weight, otherwise increasing HDP will increase egg mass of this treatment. Protein and fat from feed can be digested as the constituent ingredient for both egg yolk and egg weight. Egg mass have also been influenced by the weight of egg yolk and egg whites. Numerically, treatment using 1.5% of combination of encapsulated black cincau leaves and probiotics could increase egg mass of laying hen. This might be due to active compound in treatment that is able to increase the digestion ability and improve digestive enzyme activities, so that it can absorb the feed and use it for digestion-metabolizing. Combination of phytobiotics-probiotics would balance the non-pathogenic bacteria population in digestive system, particularly those from genus *Bifidobacterium* and *Lactobacillus*.

There was no significant effect (P>0.05) of combination of black cincau leaves and probiotics IOFC. IOFC (Rupiah / hen/ day) in this research was showed 652.20, 546.75, 559.28, 600.85, consecutively. Higher result of IOFC was better than decreasing, because relate with economically analysis, such as income for farmer. Feed in laying egg farm have the highest production cost (60-70%) (Jahan et al., 2006). The best IOFC was obtained from basal feed (T0) with Rp (Rupiah) 652.20. This situation occurs because the combination of encapsulated black cincau leaves and probiotic has more expensive price (Rp 5,498/ kg – Rp 5,788/ kg) than basal feed (Rp 5,208/ kg).

There was no significant difference (P>0.05) on egg weight on this research. Egg weight (g) result showed 61.49, 62.44, 65.34, 64.51, consecutively. It was supposed that egg weight affected by several factors, either internal or external, such as genetic, environment, feed nutrients (including amino acids and mineral). This research used balanced-amino acid (methionine and lysin), with the same composition for each treatment. The best treatment was shown in T3 (using 1.5% phytobiotic-probiotic), which was supposed that amino acids content obtained from protein content of treatment.

Table 2. Average of feed intake, hen day production, egg mass, feed conversion, income over feed cost, egg weight, yolk cholesterol content and ammonia level in 28-week-old laying hen

Variables	Treatment			
	T0	T1	T2	T3
Feed intake (g/ hen/ day)	117,62±0.67	118.34±0.95	116.68±1.94	118.05±0.22
Feed conversion	2.26±0.21	2.28±0.19	2.21±0.10	2.11±0.17
HDP (%)	84.57±6.17	84.64±6.50	83.00±3.54	89.80±2.78
Egg Mass (g/ hen/day)	53.24±4.17	52.79±4.56	53.72±3.29	57.27±4.30
IOFC (Rp/ hen/day)	652.20±30.83	546.75±116.24	559.28±85.15	600.85±106.00
Egg weight (g)	61.49±3.59	62.44±1.44	65.34±2.51	64.51±2.18
Yolk cholesterol content (mg/ 100 g)	214.59±2.83	211.71±1.65	212.55±1.33	213.32±0.52
Ammonia level (ppm)	1.42±0.26^{b}	0.80±0.03^{a}	0.81±0.08^{a}	0.80±0.18^{a}

$^{a-b}$ means different superscripts (P<0.05); followed by different lowercase letters within a row are statistically different. HDP= Hen Day Production, IOFC = Income Over Feed Cost

Effect of combination of encapsulated black cincau leaves (*mesona palustris* bl) and probiotics on yolk cholesterol content and ammonia level

The results showed that cholesterol levels of egg yolk (mg/100g) were 214.59, 211.71, 212.55, 213.32, consecutively. The result showed that there was significant effect (P≤ 0.05) on yolk cholesterol content. It is supposed that different metabolism condition of laying hen each, sample-drying method and several cholesterols distributed to meat. As numerically, T1 decreased cholesterol content on this research. This is because the content of antioxidants in treatment able in holding the cholesterol of egg yolk by inhibiting free radical formation of cholesterol. Flavonoids work in blood by

inhibiting enzymes 3-hydroxy 3-methylglutaril coenzyme a reductase (HMG CoA reductase), HMG CoA-reductase enzyme helps the formation of mevalonic acid and influencing cholesterol content (Nelson et al., 2000). Another studies showed that yolk cholesterol content carried out from serum cholesterol, i.e investigated by Kurtoglu et al. (2004) showed using 250 mg/ kg^{-1} probiotic either at 60^{th} or 90^{th} days were decreased.

There was significant difference ($P<0.05$) on ammonia level of laying's excreta. The result (ppm) was reported 1.42, 0.80, 0.81, 0.80, consecutively. It was supposed that ability of probiotic in this research to maintain small intestine of laying hen. Lactic acid bacteria express acid condition, which able to decrease ammonia gas when released. *Lactobacillus* sp. decrease the pH of excreta and number of gram-negative bacteria, it was because in acid environments would break-down metabolic processes in bacterial cells, such as metabolic formation of ATP (Adenosina Trifosfat). The condition is able to trigger the growth of non-pathogenic bacteria in the digestion tract and suppress growth of *Escherichia coli* and coliform, thus suppress production of hydrogen sulphide, which causes unpleasant-odor. Decreasing of urea content in the excreta was caused by the use of probiotics in poultry, also express an enzyme that works to hydrolyze urea into ammonia. Many studies showed that ammonia emission were decreased by probiotics, i.e *Lactobacillus salivarius* and *Pediococcus* pentosaceus on 12 d free chickens (Chen et al., 2017). Synergistic performance of encapsulated black cincau leaves and probiotics were seen able to trigger the increasing number of non-pathogenic bacteria in the intestinal villi. Otherwise, that the absorption of nutrient content occurs optimally, then the number of ammonia was decrease.

CONCLUSION

Using combination of encapsulated black cincau leaves and probiotics have not influenced on feed intake, HDP, egg mass, feed conversion, IOFC, egg weight and yolk cholesterol content, but decreased ammonia levels in excreta. Using 1.5% treatment gives best result then others.

DECLARATIONS

Consent to publish

Not applicable

Competing interests

The authors declare that there is no compete of interest in this research.

Author's contributions

The authors contributed to arrange the experimental research, determine method of research, preparation of materials method, research and data analyzed.

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Comparison of Three Lines of Japanese Quails Revealed a Remarkable Role of Plumage Color in the Productivity Performance Determination

Fadhil Rasol Al-Kafajy, Mohammed Baqur Sahib Al-Shuhaib*, Ghadeer Salah Al-Jashami, Tahreer Mohammed Al-Thuwaini

Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim 51013, Babil, Iraq

*Corresponding author's Email: mohammed79@agre.uoqasim.edu.iq

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ABSTRACT

The study was conducted to compare body weight, egg, and carcass characteristics, as well as several biochemical parameters amongst three lines of plumage color of quails, including, black, white, and brown (n= 200 each). Body weight was analyzed on a weekly basis throughout the study period (third– 13th week of age). Eggs were collected for seven consecutive weeks of sexual maturity (seventh – 13th week of age). In addition to egg quality measurements, 16 serum biochemical parameters were also determined. The brown line had exerted significantly higher values of body weight in most analyzed weeks of sexual maturity. It had given higher values of albumen height and shell thickness, as well as carcass dressing than other lines. Simultaneously, a significantly high number of eggs in the white line were observed in the most analyzed weeks. Besides, it had given higher values in terms of shell and yolk weights, as well as several carcass characteristics, such as the heart, thigh, breast, and back. The biochemical analyses had shown no significant differences amongst the analyzed populations with exception of a higher concentration of amylase in the brown line. In conclusion, our study revealed the presence of a clear superiority of the brown and white lines in terms of the meat and egg productivity, respectively. Therefore, we recommend breeders to raise brown and white lines for a better production of meat and eggs, respectively, whereas the black line has shown the least productive characteristics than other two lines throughout the study period.

Keywords: Eggs, Japanese quails, Line, Meat, Production, Serum

INTRODUCTION

Japanese quails (*Coturnix japonica*), are the smallest avian species raised for producing both meats and eggs. Several aspects account for the utility of this important bird, it provides an economic alternative to chickens. These birds attained a remarkable economic importance as an agricultural species that provide a special meat enjoyed for a unique flavor with a high nutritional value (Kayang et al., 2004). There are many reasons to encourage farmers to raise Japanese quails. Quails characterize with their low cost of maintenance, thus, poor people around the world are interested in rear quails on a commercial basis due to the lower initial investment (Jeke et al., 2018). They have a remarkable low risk rather than commercial broiler farming (Prabakaran, 2003). Furthermore, the early puberty, short generation interval (3-4 generation per year), and fewer feed requirements make these birds the most suitable and effective poultry which may boost farmers to go on in its production (Vali, 2008). In addition, there are several factors contribute for the utility of these birds in the scientific experiments, such as their resistance to diseases that associated with a high egg production which render these birds ideally suited for the scientific experimentations (Scholtz et al., 2009). In order to establish a breeding program, it is essential to estimate genetic parameters for improving the traits (Vali et al., 2005). A selection program not only affects the egg production traits but also the plumage color which depends upon various candidate genes and there has been a strong likelihood of linkage of various plumage color with quantitative traits that need to be explored (Delmore et al., 2016). Nevertheless, it is observed that most farmers confronted a problem to get a good quality commercial quail chicks (Nasar et al., 2016). Plumage

color has been reported to significantly associate with body weight and abdominal fat, and egg characteristics (Minvielle et al., 1999). Add to that, information about growth performance and plumage color mutations are insufficient to assess their use in commercial production. The approach of the desired improvement strategy could be performed by making a direct comparative study among several lines of quails (Inci et al., 2015). Noteworthy, the plumage color phenotype draws the attention of recent genotyping studies as it is the main reactive manifestation of the complicated genetic composition of Japanese quails (Badyaev et al., 2017). However, the plumage color mutants in Japanese quail have received little attention. This is due to the limited number of stock available in different countries. The performance of the blood biochemical features with bird performance is needed to select the best characteristics to improve the production and health traits (Baylan, 2017). Therefore, the present study was conducted to assess the main productive traits amongst three different plumage color lines in terms of egg and meat which are the main focus of the current study. As well, the blood biochemical analysis is also highlighted too, which may collectively build a beneficial view for breeders to select the most appropriate line for their productivity ambitions.

MATERIALS AND METHODS

Experimental design

Initially, a total of 600 Japanese quails (*Coturnix japonica*) of two weeks of age were included in the study. The included birds belong to three morphologically different lines, in terms of plumage color, namely black, or dark brown (n=200), white (n=200), and brown or wild-type, (n=200) lines (Figure 1). All three lines were purchased at two weeks of age from the directorate of agricultural research/ministry of agriculture, Baghdad, Iraq. Birds were kept under the same management conditions throughout the study at the poultry research farm of Al-Qasim green university, Iraq. Quails were housed in battery cages according to their line (Manafi, 2018). The birds were raised under the same living conditions and received the same feed. According to the National research council (1994), a standard diet containing 240g crude protein/kg and 12.1 MJ (Megajoules) of ME (Metabolized Energy)/kg as well as water was provided *ad libitum* during the rearing period. The temperature of the quails' house was around 20ºC. A lighting schedule of 16 hours light and 8 hours of darkness was applied with an intensity of five lux throughout. Farm bio-security and standard hygienic precautions were maintained strictly to prevent the outbreak of any potential infection. Sex determination was performed by observing the cloaca and breast plumage color in the sixth week (Alkan et al., 2008). On this week, all males were excluded from the study (n=377), and all females were housed individually in laying cages. Then, a total of 223 of sexually mature females were screened in the study, including black (n 54), white (n 84), and brown (n 85).

Figure 1. The investigated three quails, including black, white, and brown populations (left to right, respectively) in the present study of both sexes that photographed at the second week of age at December-2017, Babil, Iraq

Productive data recording

Due to the low level of quail's domestication (Jone et al., 1994), all data of egg production were recorded only in the period third – 13th weeks of age. Regarding live body weight, the recording of this feature was performed on weekly basis on both sexes in the period third – sixth weeks of age. Subsequently, the recording of live body weight at the sexual maturity was restricted only on the separated females (n 223). Then, both egg weight and number for each line were identified by quails' number on weekly intervals (seventh– 13th weeks). Albumen weight, albumen height, yolk weight, yolk height, shell weight, and shell thickness characteristics were measured at the 13th week of age. Subsequently, all quails were slaughtered at the end of the 13th week of age. After slaughtering, evisceration, and defeathering, carcass traits including carcass weight and other body organs, including gizzard, liver, heart, thigh, breast, neck, back, wings were measured in carcasses.

Biochemical data recording

After slaughtering birds at the 13^{th} week of age, blood samples were collected by cervical dislocation and were then decapitated. Subsequently, blood sera were initially prepared according to the procedure mentioned by Scholtz et al. (2009). The main serum biochemical parameters, including albumen, total protein, Globulin (GLOP), Albumen/Globulin (A/G), Total Bilirubin

(TBIL), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Amylase (AMYL), Creatinine (CREA), Creatine Kinase (CK), Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL), were recorded according to the manufacturer's instructions (Chengdu Pulitai Biological techn. Co., Hi-tech, China). All biochemical experiments were performed in a fully automated hematological analyzer (Methic 18 Vet, Orphee, France).

Statistical analysis

The collected data were analyzed by general linear model procedure of SAS statistical package software (Statistical Analysis System, 2012). Least significant differences for a parameter were used to calculate the significant difference amongst three lines of quail. The following general linear statistical model was used to analyze the different parameters:

$Y_{ij} = \mu + t_i + e_{ij}$,

Where

Y_{ij} is the dependent variable of the experiment;

μ is the overall mean;

t_i is the effect of *ith* type (i = 1-3);

e_{ij} is the error term specific to each record.

The differences between the means were statistically estimated by ANOVA – Duncan's test. All values were expressed in mean standard error (SE) using a significant level of $P < 0.05$ and $P < 0.01$.

Ethics approval

All procedures involving animals were approved by the animal care and use committee at the respective university where the experiments were conducted (dated 11-22-2017, Decision No. 134).

RESULTS

Body weight

Regarding the first four weeks of raising (third week – sixth week), in which both sexes were recorded, our results had revealed no superiority for any one of the three lines. Subsequently, a remarkable difference was observed regarding the overall superiority of brown lines after separating both sexes from each other. With exception to the sixth week, in which the black line had higher body weight values, our results had shown significantly higher values ($P< 0.01$) for brown line females in most of the analyzed weeks than other two studied lines, black and white, respectively (Table 1).

Egg number and weight

The present study found significantly higher values for egg number and weight ($P<0.01$) in the white line in almost all studied weeks (Table 2). The only one exception for this observation is the higher values that were seen in the eighth week in which the brown line had significantly ($P< 0.01$) superseded the other two lines in both egg weight and number. However, a total clear superiority of white line was observed in terms of egg weight and number.

Egg external characteristics

Upon examining the effect of line differences on other recorded parameters, such as albumen weight and height, yolk weight and height, shell weight and thickness, an obvious competition was observed between both white and brown lines. This competition was presented by the observation of higher values for albumen height ($P< 0.01$) and shell thickness ($P< 0.05$) in the brown line, while the white line had exhibited higher values for yolk weight and shell weight ($P< 0.01$ each) (Table 3). Simultaneously, no significant differences were observed in the other analyzed traits, such as egg weight, and yolk height. The proportion of yolk weight in white line eggs is significantly higher than those found in the other two lines ($P< 0.01$). This observation was accompanied by a parallel high shell weight found in the same line.

Carcass traits

It was observed from the result of this study that color variation had a significant effect on several carcass traits. This observation was exhibited in the white line as it had shown highly significant values ($P<0.01$) in the thigh, breast, and back than other two lines (Table 4), while the brown line had given higher values in carcass dressing traits.

Biochemical criteria

The results of biochemical analyses are presented in table 5. With one exception observed in the amylase levels, in which highly significant values ($P< 0.01$) of this enzyme was recorded in the brown line, other serum biochemical parameters had shown no significant differences ($P\geq 0.01$) amongst other serum parameters, including albumen, total protein, GLOB, A/G, TBIL, AST, ALT, ALP, AMYL, CREA, Urea, CK, TC, TG, HDL, and LDL.

Table 1. Mean live body weight in grams recorded for different production weeks in three lines of quails. This experiment is extended from December-2017 to March-2018, Babil, Iraq

Variables / Sex	Line	Mean ± SE third week M+F	fourth week M+F	fifth week M+F	sixth week M+F	Seventh week F	eighth week F	ninth week F	tenth week F	11th week F	12th week F	13th week F
BW	Black	84.09^{b}±14.1	124.12^{b}±14.1	168.88^{ab}±14.1	226.41^{a}±14.1	194.71^{b}±14.1	218.492^{ab}±14.1	245.680^{a}±14.1	252.9^{a}±14.1	261.86^{ab}±14.1	272.60^{ab}±14.1	283.11^{ab}±14.1
	White	86.04^{a}±14.1	124.02^{b}±14.1	163.4^{b}±14.1	193.4^{b}±14.1	195.4^{b}±14.1	202.5^{b}±14.1	235.7^{b}±14.1	248.5^{b}±14.1	256.2^{ab}±14.1	263.9^{b}±14.1	267.6^{b}±14.1
	Brown	87.1^{a}±14.1	128.9^{a}±14.1	170.2^{a}±14.1	199.1^{b}±14.1	215.9^{a}±14.1	229.6^{a}±14.1	246.04^{a}±14.1	248.1^{b}±14.1	274.1^{a}±14.1	287.7^{a}±14.1	294.0^{a}±14.1
Level of significance		NS	NS	NS	**	**	**	**	NS	**	**	**

Significant differences in means represented by different letters in the same column. BW; Body Weight, SE; Standard Error, *; (P<0.05), **; (P<0.01), NS; Non-Significant, M; male, F; female

Table 2. Mean egg numbers and weights in grams, for different production weeks in three lines of quails. The experiment is extended from January to March 2018, Babil, Iraq

Phenotypes / Traits	Line	Mean ± SE Seventh week	Eighth week	Ninth week	Tenth week	11th week	12th week	13th week
EN	Black (54)	39.685a±1.961	35.963b±1.915	25.481b±3.321	31.056b±2.401	36.148ab±2.030	28.685b±1.500	19.130b±0.984
	White (84)	38.548a±1.573	38.548ab±1.536	59.250a±2.663	55.798a±1.925	52.429a±1.627	38.048a±1.203	31.560a±0.789
	Brown (85)	36.800a±1.563	53.800a±1.527	32.400ab±2.647	38.400ab±1.914	32.800b±1.618	32.200ab±1.196	22.600ab±0.785
Level of significance		NS	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**
EW	Black (54)	94.348b±8.036	341.389ab±16.573	240.463b±34.003	340.093b±24.351	361.574ab±23.203	282.500b±15.897	216.111b±10.339
	White (84)	262.536a±6.444	262.536b±13.288	611.190a±27.263	567.952a±19.524	577.107a±18.604	383.631a±12.746	342.679a±8.290
	Brown (85)	229.000ab±6.406	521.400a ±13.209	321.000ab±27.102	403.000ab±19.409	342.000b±18.494	323.400ab±12.671	259.000ab±8.241
Level of significance		**	**	**	**	**	**	**

EN; eggs number, EW; eggs weight, SE; Standard Error, *; (P<0.05), **; (P<0.01), NS; Non-Significant, significant differences in means represented by different letters in the same column

Table 3. External and internal egg quality traits of 13-week-old in quail lines, the studied traits include albumin weight, albumin height, yolk weight, yolk weight, shell weight and shell thickness. The experiment is performed at March-2018, Babil, Iraq

Traits / Lines	Mean ± SE					
	AW (g)	AH (mm)	YW (g)	YH (mm)	SW (g)	ST (mm)
Black (54)	11.405±0.163	31.235±0.146b	3.718±0.135b	21.824±0.167	1.177±0.040b	0.156±0.055b
White (84)	11.434±0.131	32.677±0.117ab	4.372±0.108a	21.966±0.134	1.348±0.032a	0.168±0.044ab
Brown (85)	11.420±0.130	33.320±0.116a	3.909±0.107ab	21.762±0.133	1.236±0.032ab	0.288±0.044a
Level of significance	NS	**	**	NS	**	*

AW; albumin weight, AH; albumin height, YW; yolk weight, YH; yolk height, SW; shell weight, ST; shell thickness, SE; Standard Error, * ;(P<0.05), **; (P<0.01), NS; Non-Significant, significant differences in means represented by different letters in the same column. AW; albumin weight, AH; albumin height, YW; yolk weight, YH; yolk height, SW; shell weight, ST; shell thickness

Table 4. Comparison of the average carcass characteristics of 13-week-old in quail lines. The experiment is performed at March-2018, Babil, Iraq

Carcass traits / Lines	Quail lines (Mean ± SE)			Level of significance
	Black (n=54)	White (n= 84)	Brown (n= 85)	
Carcass weight (g)	114.982 ± 5.188 a	130.570 ± 5.188 a	116.122 ± 5.188 a	NS
Gizzard % of carcass	5.046 ± 0.609 a	5.262 ± 0.609 a	4.656 ± 0.609 a	NS
Liver % of carcass	7.620 ± 0.264 a	8.018 ± 0.264 a	6.234 ± 0.641 a	NS
Heart % of carcass	1.718 ± 0.641b	2.084 ± 0.641ab	1.504 ± 0.264 b	**
Thigh % of carcass	34.256 ± 1.593 b	40.274 ± 1.593a	35.842 ± 1.593 ab	**
Breast % of carcass	49.698 ± 2.345 b	57.942 ± 2.345 a	51.576 ± 2.345 ab	**
Neck % of carcass	6.344 ± 0.549 a	6.410 ± 0.549 a	5.302 ± 0.549 a	NS
Back % of carcass	12.974 ± 1.477 ab	13.694 ± 1.477 a	8.276 ± 1.477 b	**
Wings % of carcass	9.226 ± 1.441 a	10.326 ± 1.441 a	12.836 ± 1.441 a	NS
Carcass dressing (%)	247.476 ± 15.198 ab	205.152 ± 15.198 b	256.116 ± 15.198 a	**

SE; Standard Error, *; (P<0.05), ** ;(P<0.01), NS; Non-Significant, significant differences in means represented by different letters in the same column

Table 5. Serum biochemical parameters (mean ± standard error) of 13-week-old in quail lines. The experiments are performed at March-2018, Babil, Iraq

Parameters / Lines	Quail lines (Mean ± SE)			Level of significance
	Black (n=54)	White (n=84)	Brown (n=85)	
Albumin (g/L)	12.133 ±1.475	9.000±1.475	11.533±1.475	NS
Total protein (g/L)	30.400 ± 2.246	28.000 ± 2.246	33.367 ± 2.246	NS
GLOB(g/L)	30.933 ± 0.038	30.100 ± 0.038	32.633 ± 0.038	NS
A/G	11.617 ± 1.232	15.000 ± 1.232	10.540 ± 1.232	NS
TBIL(μmol/L)	127.033 ± 6.111	112.867 ± 6.111	92.600 ± 6.111	NS
AST (U/L)	302.000 ± 49.879	257.333 ± 49.879	268.000 ± 49.879	NS
ALT (U/L)	7.000 ± 1.673	6.667 ± 1.673	9.667 ± 1.673	NS
ALP(U/L)	94.333 ± 40.159	99.000 ± 40.159	93.667 ± 40.159	NS
AMYL(U/L)	128.333 ± 12.597	94.000 ± 12.597	262.000 ± 12.597	**
CREA(μmol/L)	30.833 ± 3.340	32.867 ± 3.340	39.667 ± 3.340	NS
Urea(mmol/L)	1.240 ± 0.014	1.243 ± 0.014	1.003 ± 0.014	NS
CK(U/L)	1035.333 ± 38.679	926.333 ± 38.679	810.333 ± 38.679	NS
TC (mmol/L)	7.530 ± 0.770	7.910 ± 0.770	5.477 ± 0.770	NS
TG (mmol/L)	6.343 ± 0.036	6.047 ± 0.036	6.803 ± 0.036	NS
HDL (mmol/L)	1.947 ± 0.030	1.720 ± 0.030	1.423 ± 0.030	NS
LDL (mmol/L)	5.583 ± 0.479	6.190 ± 0.479	4.053 ± 0.479	NS

Significant differences in means represented by different letters in the same column, SE; Standard Error, *; (P<0.05), **; (P<0.01), NS; Non-Significant, GLOB; globulin, A/G; albumin: globulin, TBIL; total bilirubin, AST; aspartate aminotransferase, ALT; alanine transaminase, ALP; alkaline phosphatase, AMYL; amylase, CREA; creatinine, CK; creatine kinase, TC; total cholesterol, TG; triglyceride, HDL; high density lipoprotein, LDL; Low density lipoprotein

DISCUSSION

In the present study, a comparative evaluation of three lines of quails was performed to assess the best one in terms of egg and meat as well as biochemical characteristics. Accordingly, several measurements were observed, such as body weight, egg number, weight, external characteristics, carcass traits, as well as biochemical criteria. These cumulative measurements can potentially provide a concrete basis for choosing the appropriate egg/meat productive line that suits the desired breeders' demands.

Body weight

Several researchers were relatively agreed with present findings of body weights values as they indicated a relatively high body weight for the brown line than other analyzed lines (Petek et al., 2004; Minvielle et al., 2005; Yilmaz and Çağlayan, 2008; Sogut et al., 2015). These values are in agreement with the reports that stated the body weights were significantly influenced by different types of color mutants or varieties of quails (Rahman et al., 2010). On the other hand, the present study showed an obvious tendency of weight superiority toward the brown line that was clearly observed only after sexual maturity. This observation indicated that there is a potential interaction between gender type, plumage color, and body weight as the superiority of the brown line is highlighted only in females after two sex's separation. In agreement with our results, several reports were suggestive that the body weight was affected by the gender of the bird (Khaldari et al., 2010; Akbarnejad et al., 2015). However, the higher body weight values for brown line indicates a preferable tendency for the meat type quail production for the brown line of quail other than the two studied lines.

Egg number and weight

The present study revealed a remarkable role for plumage color in the egg number and weight characteristics. This observation came in line with a series of accumulated results that found significant differences in egg weight among different lines of quail (Ashok and Reddy, 2010). Another confirmation of this finding came from other reports that observed that egg production had significantly differed by the different lines of quails (Soliman et al., 2000; Rahman et al., 2010). However, the determination of the best plumage color line in terms of egg productivity is quite controversial among the published data. In contrast to our present results, in which we have observed a clear superiority of the white lines in both egg number and weight, Yilmaz and Çağlayan (2008), have revealed that the eggs of the white line had weighed significantly less than those of the other groups, while no significant differences between these lines and other studied lines were observed. Similarly, Ashok and Reddy (2010) have shown that the brown line has exhibited high values of egg weight, while the black lines have shown significant ($P<0.05$) superiority in terms of egg number. Conversely, Faruque et al. (2013) have reported that the egg weight of white line has higher percentage values than the other lines. Furthermore, any possible correlation between plumage color and egg characteristics was refuted by Farghly et al. (2015). No easy explanation for these differences is feasible, but the variation in the environmental conditions and sampling error due to limited sample size could not be excluded from such explanation (Prado-Gonzalez et al., 2003). Regarding live body weight and egg productivity, the present study indicated the presence of a prominent negative correlation between body weight and egg production. This correlation is obviously seen in the brown and white line as the brown line exhibited higher values of body weight and parallel lower values for egg productivity in comparison with the white counterpart that showed the opposite characteristics. This negative correlation has been widely confirmed in several quail variations (Silva et al., 2013; Baylan, 2017).

Egg external characteristics

Up to our knowledge, there was no published data revealed by other researches so as to compare our results with. However, the present study has clearly observed that the proportion of yolk in the white line eggs is larger ($P< 0.01$) than those in other lines, which may be correlated with their higher values of the egg weight (Ahn et al., 1997).

In the case of the shell thickness and albumen height, significantly higher values were exerted in the brown line. This observation was not revealed by Inci et al. (2015) who found that there were no significant differences among the several quail lines with regard to shell thickness. However, as well as the albumen height is an important trait and it is feasible to improve egg quality through, the eggs with higher albumen height tend to have better internal egg quality (Khawaja et al., 2013). This observation potentially indicates that the brown line has presented a better albumen quality than white and black lines, respectively. However, the present

data were not in agreement with Yilmaz and Çağlayan (2008) who stated through recording the egg-shape index characteristics the absence of any significant differences among the studied lines of Japanese quails.

Carcass traits

Strain type is one of the factors affect carcass quantity and quality in Japanese quail (Kumari et al., 2008). Furthermore, the growth performances of carcass traits of Japanese quails raised under different conditions were compared (Inci et al., 2016). However, several variations were recorded in the studied lines in several carcass traits, which indicate an effective role for these variable lines in such characteristics. The observed variations of the present study appeared in the superiority of the white line in several internal organs values, including heart, thigh, breast, and back. Our results were in agreement with a recent study that reported an obvious superiority of white line in several productive features including carcass weight (Nasr et al., 2017). Simultaneously, our finding exhibits an obvious superiority for the brown line in terms of carcass dressing, then the other two studied lines. The recorded higher values of carcass dressing characteristics of the brown line that observed by Inci et al. (2015), came in line with our findings of this wild-type line. However, the clear superiority of the brown line in terms of carcass dressing may be correlated with its significantly higher values of body weight. Thus, this observation gives us another indication for the higher tendency of the brown line for meat production.

Biochemical criteria

The biochemical analyses are valuable tools for evaluating traits in breeding for high productivity and as indicators for the health of birds (Karesh et al., 1997). With few exceptions, the present study found no significant differences amongst the studied three lines in almost all biochemical criteria. Unfortunately, a few published data regarding biochemical records of this bird were available (Scholtz et al., 2009), which limit the comparison issue. This limitation may be due to the small size and the highly mobile nature of this bird that increase the technical difficulty of the sample collection (Sokół et al., 2015). Nevertheless, the present study has provided comparable concentration with regard to albumen, total protein, AST, urea, TC, and TG and similarly higher concentrations regarding ALT and CK than the concentrations observed by other related reports in quails (Saki et al., 2017). The reason behind these variations is unknown but it could be attributed to the type of the population, and the method of estimation that may deviate the observed data to some extent (Falconer and MacKay, 1996). However, the present results have come in line with Khawaja et al. (2013), who found a non-significant ($P > 0.05$) difference in blood glucose, triglyceride, cholesterol, calcium, protein, uric acid and ALP values among all chickens. The present study has suggested that the genotype of plumage color has intervened with the amylase level among the three analyzed lines of quail. This intervention was presented by the presence of significantly higher values of amylase level in the brown line. However, this observation may suggest a potential role for amylase concentration in the current comparative study among three genotypically different lines of quails. This suggestion may be aided by Brzęk et al. (2013), who have shown that the pancreatic enzymes in birds are regulated under a strict genetic control.

CONCLUSION

In the light of the obtained results, it can be stated that both white and brown lines had, in general, higher values of productivity compared with the black line. It can be determined from the present findings that the performance of white quails was superior in terms of egg production, and several carcass traits, while the brown line has been characterized with higher body weight and carcass dressing, as well as several biochemical parameters and increased egg-shell thickness. Since the white line was clearly shown highly significant values in terms of egg number and weight, the present study recommends it in terms of high egg production purposes. Similarly, this study recommends the brown line in the meat type production purposes. In contrast to brown and white, the black line was not currently recommended in terms of egg and meat production.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

All the authors have made a substantive contribution to the study.

Consent to publish

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

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A Review on Potential of Glutamate Producing Lactic Acid Bacteria of West Sumatera's Fermented Food Origin, as Feed Additive for Broiler Chicken

Vebera Maslami[1], Yetti Marlida[2*], Mirnawati[2], Jamsari[3], Yuliaty Shafan Nur[2], Frederick Adzitey[4] and Nurul Huda[5]

[1]*Department of Agricultural Science, Faculty of Agriculture, Andalas University, 25163, Indonesia*
[2]*Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, 25163, Indonesia*
[3]*Department of Crop Science, Faculty of Agriculture, Universitas Andalas, 25163, Indonesia*
[4]*Department of Veterinary Science, University for Development Studies, Box TL 1882, Tamale, Ghana*
[5]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia*

*Corresponding author's Email: yettimarlida@ansci.unand.ac.id; ORCİD: 0000-0001-5395-1297

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ABSTRACT

Increasing broiler populations must be supported by cheap and high quality feed. Improving the quality of feed can be done by adding feed additives. Glutamate is a non-essential amino acid that can be used as a feed additive in the form of flavoring agents in broiler feed which functions as a neurotransmitter of taste, basic structure of proteins, and in metabolism of the body. Lactic Acid Bacteria (LAB) are one of the microbes that are considered faster and safe in producing glutamate. Fermented foods of West Sumatera, Indonesia origin serve as sources of LAB include dadih (fermented milk), asam durian (fermented durian), ikan budu (fermented fish) and tapai (fermented rice and cassava). The West Sumatra's fermented foods are potential sources of glutamate. Supplementation of glutamate in broiler diet can increase body weight, protein digestibility, reduce faecal ammonia and improve carcass quality (improve umami taste, and reduce bruises and abdominal fat).

Key words: Carcass quality, Feed additive, Fermented food, Glutamate, Lactic acid bacteria, Performance

INTRODUCTION

Broiler is a meat-producing animal that has enough potential to meet people's needs for animal protein. This is because broiler meat is relatively inexpensive and easily obtained compared to other animal proteins. High broiler populations must be supported by high quality feed to improve the performance and quality of broiler carcasses. One important component of feed needed to increase growth and feed efficiency is feed additive. According to Slyamova et al. (2016), feed additives has function in improvement of immune system against disease attacks and effects of stress, increase appetite, stimulate growth, and increase the production of meat and eggs. Feed additives can be flavoring agents, antibiotics, enzymes, antioxidants, hormones, probiotics and anticoccidials (Lesson and Summers, 2001).

One of the feed additives that can be used in animal feed is glutamate. Glutamate is a feed additive that serves as flavoring agent which helps to increase feed consumption. According to Fujimura et al. (2001), glutamate functions as a neurotransmitter for taste, where glutamate is serves as umami, flavors or savory. Besides functioning as a glutamate flavoring agent, it functions as a protein building block, a substrate in the synthesis of protein, precursor of glutamine and helps the body's metabolism (Young and Ajami, 2000).

Giving glutamate to broilers has been carried out by several researchers. According to Berres et al. (2010), administering glutamate to broilers decreases abdominal fat, improves meat examination by reducing red bruises on carcasses and increasing meat protein content. The administration of 0.75% glutamate in feed increases muscle free glutamate and improves the taste of meat (Fujimura et al., 2001; Imanari et al., 2008). Furthermore, administration of glutamate improves broiler performance, reduce the feed conversion ratio, affect the length of intestinal villi, reduce raw ration protein and reduce faecal ammonia (Zulkifli et al., 2016; Ribeiro et al., 2015; Bezerra et al., 2016).

The sources of glutamate are animals, plants and microbes. Microbes are mostly used to produce glutamates probably because it is easier, faster, safer and relatively inexpensive compared to other sources. Glutamate production can be done by fermentation or by chemical processes. At present, most glutamates are produced by fermentation because it is superior to chemical processes. The fermentation process produces L-glutamate while the chemical process produces racemic glutamate (D- and L-glutamic acid) (Sono, 2009). The difference of L-glutamate and D-glutamate is their uses in the body. According to Wijayasekara and Wansapala (2017), L-glutamate is the form glutamate is used in the body.

Lactic Acid Bacteria (LAB) are examples of microbes that produce glutamate. According to Lucke (2000), LAB is one of the gram-positive bacterium that produces glutamate which is considered safe and environmentally friendly. A number of LAB isolated from different sources have been reported to produce glutamate. These include LAB from tapaipulut (Ishak et al., 2017), *Lactobacillus plantarum* MNZ from fermented food (Zareian et al., 2012), *Bacillus spp* from vegetable protein (Lawal et al., 2011) and *Lactobacillus* from skim milk (Zalan et al., 2010).

West Sumatera is rich in the production of fermented foods which are sources of LAB. Fermented foods in West Sumatera that are widely consumed contain dadih (fermented milk), asam durian (fermented durian), ikan budu (fermented fish) and tapai (fermented rice and cassava) (Mustopa and Fatimah, 2014). Purwandhani et al. (2018) isolated LAB from dadih of West Sumatera origin. Yusra (2014) reported of *Bacillus spp* from ikan budu in Pariaman. Furthermore, Chuah et al. (2017) found six LAB strains in asam durian (*Fructobacillus durionis, Lactobacillus plantarum, Lactobacillus fructivorans, Leuconostoc dextranicum, Lactobacillus collinoides and Lactobacillus paracasei*) and Sujaya et al. (2001) *Weissella spp* in tapai. There is high diversity of fermented foods in West Sumatera, therefore, it is necessary to explore their nature and potentials as sources of LABs capable of producing glutamate, which can serve as feed additive for broilers.

Food Fermentation from West Sumatra

Fermentation was carried out in the past based on non-scientific studies and much was not known about the role of microbes in changing food characteristics. It was based on traditional techniques of storing and handling food ingredients which turns out to produce new food products that are different from the original ones. The goal of initial food fermentation was to preserve seasonal foods that were easily damaged. During fermentation, the digestibility and nutritional quality of foods are improved. There is the addition of vitamins, and essential amino acids and fatty acids during fermentation (Steinkraus, 2002).

Indonesia has many fermented foods scattered throughout the region. Some fermented foods in Indonesia include: Brem (fermented rice) from Madiun and Wonogiri, danke (fermented buffalo milk) from Sulawesi, lemea (fermented bamboo) from Bengkulu, oncom (fermented tofu waste) from West Java, pakasam and wadi (fermented fish) from various regions of Indonesia, tempe (fermented soybeans) from various regions of Indonesia, gatot and growol (fermented dried yam) from Yogyakarta, Tempoyak (fermented durian) from various regions of Indonesia and urutan (pig fermentation) from Bali (Nuraida, 2015). Even though, the same fermented foods may be produced in different regions, the manufacturing process are slightly different. West Sumatra also has a lot of fermented foods, among them are dadih, asam durian, ikan budu and tapai.

Dadih

Dadih is a fermented food from milk which is a potential functional food and a source of probiotic. Dadih processing is still done traditionally without any standard processing procedure. Curds are made from buffalo milk poured into a bamboo tube to ferment naturally for 24 to 48 hours at room temperature. The natural fermentation process in making curds involves various types of microbes found on the surface of the inner bamboo tube, the surface of the cover leaves and from buffalo milk used. According to Hasim et al. (2017), dadih produced in West Sumatra are made from buffalo milk by relying on natural microbes as the inoculum. The bamboo is usually covered with taro leaves, banana leaves, plastic, or left without a cover, depending on the region (Maskiyah and Broto, 2011).

Isolation and use of LAB in dadih have been reported by several researchers. In a study by Surono (2003), LAB in curds were dominated by *Lactococcus* bacteria consisting of *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Lactococcus brevis*, Lactococcus *casei*, *Lactoscoccus plantarum*, and *Enterococcus faecium*. Pato et al. (2005) found the following LAB, *Lactobacillus casei* subsp *casei*, *Leuconostoc paramesenteroides*, *Enterococcus faecalis* subspecies

liquefaciens, and *Lactococcus lactis* subsp *lactis* in dadih.

Asam durian

Asam durian is a fermented product made from durian. Fermented foods are found in several regions (West Sumatra, Jambi and Riau) of Sumatera, Indonesia. The process of making asam durian and the name given to it differ among each region. In Jambi of Indonesia, durian fermentation is called tempoyak while in West Sumatera and Riau it is known as asam durian. Fermentation of durian is done by the Jambi community by adding salt (Anggraini and Widawati, 2015). LAB found in salt-containing asam durian includes *Lactobacillus species*, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Weissella paramesenteroides* (Yuliana and Dizon, 2011).

Asam durian found in West Sumatera Indonesia has differences from other regions. The manufacturing process is spontaneous fermentation, by natural microorganisms found in nature without adding salt during incubation. Six types of bacteria have been identified in asam durian. They are: *Lactobacillus plantarum, Pediococcus acidilactici, Leuconostoc mesentroides, Staphylococcus saprophyticus, Lactobacillus curvatus*, and *Micrococcus variance* (Hasanudin, 2010).

Ikan budu

Ikan budu is a product made from fermented fish and mainly from coastal areas of Pasaman Regency and Padang Pariaman in West Sumatera, Indonesia. In Indonesia, it is found only in West Sumatra. This fermented food product is made from large sea fish and has white meat. Example of fishes used for making budu are mackerel (*Scomberomorus guttatus*) and chamfer (*Chorinemus lyson* L). Budu fish has a distinctive aroma, not rotten, non-rancid and soft-textured. Yusra et al. (2014) found that *Bacillus* and *Micrococcus* species were involved in ikan budu fermentation.

Malaysia also produce budu but the fermentation process and type of fish used is different from that of West Sumatra. Budu from Malaysia appear as thick brown, and it is prepared from salted raw anchovy (*Stolephorus spp*.) and stored in a large concrete tank at 30-40^{o}C for 6-12 months (Sim et al., 2015). Sim et al. (2015) identified *Pediococcus* (9.4%), *Candida* (8.0%), *Micrococcus* species (28.7%), *Staphylococcus* (26.7%), *Lactobacillus* (6.7%), *Saccharomyces* (4.0%), and *Lactococcus* (2.7%) from Malaysian budu. Fujimura et al. (2012) also isolated LABs from Malaysian budu.

Tapai

Tapai is one of Indonesia's traditional foods produced from the fermentation of carbohydrate foods such as cassava and sticky rice. Tapai fermentation process takes place because of the activities of several types of microorganisms such as bacteria, yeast and fungi. Tapai is produced by fermenting cassava with yeast, LAB, and amylolytic bacteria (Sujana, 2001). Different from other places, glutinous tapai from Indonesia, especially from West Sumatra, uses black glutinous rice stored in containers coated with banana leaves, while in other areas guava leaves (*Syzygium*) or rubber (*Hevea brasiliensis*) are used. Suhartatik et al. (2014) isolated LAB such as *L. mesenteroides, L. pentosus, L. brevis*, and *L. plantarum*, from Tapai. Also, Maslami et al. (2017) isolated *Lactobacillus sp* from tapai of West Sumatera origin. In Malaysia, different ingredients and fermentation processes are used in tapai preparation.

Tapai from Sabah, Malaysia is an alcoholic drink but not food (Campbell-Platt, 2000). In Sabah, the basic ingredients for making tapai are rice, cassava, pineapple and corn which are fermented using cultural starter from Sasak (Chiang et al., 2006). Tapai is also produced at Peninsula, Malaysia. The processing method employed in Peninsula, Malaysia is similar to how tapai is prepared in Singapore and Brunei, that is, they all carry out fermentation in banana leaves (Campbell-Platt, 2000). LAB found in tapai of Sabah origin include *L. brevis*, *L. plantarum*, *P. pentosaceus*, *L. lactis* and *L.s paracasei* (Chiang et al., 2006). *L. casei* is also involved in the fermentation process of tapai of Malaysia origin (Adnan and Tan, 2007).

LAB Producing Glutamate

Research on glutamate producing microbes has been carried out by several researchers. Glutamate producing microbes isolated from various sources can be seen in table 1.

Effects of glutamate on performance and quality of broiler carcasses

Glutamate plays an essential role in the metabolism and increases feed consumption. The purposes and dosages for the administration glutamate in broiler is presented in table 2.

Table 1. Glutamate producing lactic acid bacteria and their source of isolation

Researcher	Source	Bacteria	Production
Tarek and Mustafa (2009)	Egyptian infants	*Lactobacillus paracasei*	68.78 mg/l.
Zareian et al. (2012)	Fermented soybean	*Lactobacillus plantarum*	489 Mmol/ L
	Fermented durian		20 Mmol/ L
	Fermented tapioca		59 Mmol/ L
	Fermented rice		65 Mmol/ L
	Fermented shrimp		11 Mmol/ L
	Fermented fish		106 Mmol/ L
Fudou et al. (2002)	Soils and vegetable	*Corynebacterium.*	-
Lawal et al. (2011)	Vegetable protein	*Bacillus subtilis*	8.4 mg/ml
		Bacillus pumilus	8.2 mg/ml
		Bacillus licheniformis	6. 4 mg/ml
		Bacillus polymyxa	6.2 mg/ml

Table 2. Purpose and dosage of glutamate administration

Researcher	Purpose	Glutamate dose
Stilborn and Moran (2010)	Increase performance and anticoccidial	1.75 %
Zulkifli et al. (2016)	Growth and physiological anti-stress on broiler chicks	1%
Joshua et al. (2015)	Anti-stress due to heat stress in the tropics	Stater (0.50% - 1 %) Finisher (0.50% - 1 %)
Ribeiro et al. (2015)	Improve performance and immune system	0.4 %
Shakeri et al., (2014)	Anti-stress on broiler chicks which are affected by heat stress	0.50 %
Berres et al. (2010)	Improve feed efficiency	Age 1-7 days (0.13% and 0.26%) Age 8-21 days (1.46%, 1.66% and 3.13%) Age 22-35 days (1.24%, 1.40% and 3.50%) Age 36-42 days (1.04%, 1.18% and 3.22%)
Khadiga et al. (2009)	Increase performance	1%
Bezzera et al. (2015)	Efficient use of feed protein	Age 1-7 days (crude protein 20.29% and glutamate 3.41) Age 8-21 days (crude protein 18.99 and glutamate (3.32) Age 22-35 (crude protein 16.24 and glutamate 2.64 Age 36-45 (crude protein 17.04 and glutamate 2.96)

Improve performance

Glutamate is known as a conditional or non-essential amino acid. Glutamate binds with other amino acids to form protein structures (Berres et al., 2010). Weight gain in 21 day broilers given an additional 1% of glutamate supplements was significantly higher (11%) than those fed without 1% glutamate addition (Bartell and Batal, 2007). The positive effect of glutamate on broiler chickens have been reported by other researchers. According to Bazzera et al. (2014), broilers fed on glutamate containing feed (1.66% up to 3.13%) performed better than those fed without glutamate supplemented feed during heat stress. Feeding broilers on glutamate increases broiler body weight and the deposition of connective tissues at growth phase (Zhang et al., 2008). According to Zulkifli et al. (2016) administration of 1% glutamate in rations can increase body weight, reduce feed consumption, improve feed conversion, and reduce death. Administration of 2% glutamic acid reduces crude protein of ration and can improve performance and reduce faecal ammonia (Ribeiro et al., 2015; Bezerra et al., 2016).

Glutamate is essential for the maintenance of intestinal mucosa and seen as an efficient source of a

non-specific nitrogen source. This is because its energy source is for rotating the mucosa, through ATP which results from the citric acid cycle (Berres et al., 2010). The synthesis of nitric oxide in some tissues (e.g. brain tissue) are regulated by glutamate (Li et al., 2007). Glutamate also act as precursor for glutathione. Glutathione is an important compound responsible for elimination of oxidants and immune system modulation (Li et al., 2007). According to Silva et al. (2001), adding L-glutamate (5, 10 and 15%) to broiler feed takes care of protein deficiencies in rations with efficiencies greater than 10%.

Carcass quality

Glutamate is a vital amino acid responsible for flavor enhancement and as feed additive (Fujimura et al., 2001). In its free form or unbound to other amino acids in protein, glutamate has a flavor enhancing effect (Yamaguchi and Ninomiya, 2000). This free glutamate functions effectively as a flavor generator and plays a role in improving delicacy. Free glutamate also known as an important taste component of meat and contributes to meat tastes (umami, brothy taste and delicious). The chicks of poultry contains more free glutamate than pork and beef (Kato and Nishimura, 1987). Pork also contain more free glutamate than beef (Kato and Nishimura 1987). The hydrolysis of protein by the supplementation of heat during cooking causes glutamate to release protein molecule that can causes umami taste (Khropychheva et al., 2009). However, when protein molecules bind with glutamate, umami taste is not released and make the protein source tasteless (Khropychheva et al., 2009).

Glutamate supplementation has a significant effect on broiler carcass quality. Low glutamate content in broiler meat will result in less glutamate being stored in muscle fibers. Glutamate level can become low because it is widely used for metabolism and as an energy source by intestinal cells. When free glutamate is present in broiler meat its umami taste reduces (Tang et al., 2009). Glutamate content in broiler meat can be increased by adding glutamate to broiler feeds.

Glutamate can also improve the quality of broiler carcasses. According to Ajinomoto (2007) and Bezerra et al. (2015) glutamate can increase meat protein, reduce meat fat, reduce meat bruises and improve meat flavor. Meat protein is increased because glutamate is a precursor of several amino acids (Berres et al., 2010). In addition, a decrease in meat fat is caused by foods that contain good source of amino acids and less energy for fat accumulation. Moran and Stilborn (1996) reported that, there was a decrease in bruises and thigh deformation and an increase in connective tissue deposition at growth phase of broilers fed by glutamate supplemented feed.

CONCLUSION

Glutamate can improve carcass traits, performance and quality of meat. One of the microbes that can produce glutamate is LAB. A potential and unexplored LAB source, that produce the glutamate found in fermented foods in West Sumatera, Indonesia.

DECLARATIONS

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Competing interest

The authors declare that they have no competing interests.

Author's contribution

All the authors have made contribution to the study.

Consent to publish

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

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Molecular Survey and Characterization of H5N8 Isolates during 2016-2017 on Egypt

Mahmoud El-Saied Sedeik*, Nahed Ahmed Elshal, Ashraf Mahmoud Awad and Nehal Kandil

Department of Avian and Fish diseases, Alexandria University, Egypt

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

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ABSTRACT

Avian influenza (AI) disease still threat poultry industry in Egypt causing great economic losses. In order to identify and characterize the agent of suggestive clinical cases of AI disease, 28 flocks showing clinical signs suspected to be due to AI infections have been investigated. By slide Haemagglutination (HA), the positive samples were 14/28 and concerning the results of real time- reverse transcriptase polymerase chain reaction (RRT-PCR), 2/14 samples were positive to AI H5, 7/14 to New castle disease virus (NDV), 1/14 to H9 and 4/14 co-infected (2 samples had NDV + AI H5 and others had NDV + AI H9). These positive PCR samples were subjected to further characterization by genotyping and sequencing analysis. The two isolated of H5 AI strain were classified to H5N8 which, related to Russian strains (clade 2.3.4.4) and the genetic analysis approved little relationship between these two H5N8 strain and the commercial AI vaccines with percent (80- 91.7%). So, the researchers should have more monitoring for these viral diseases with effective biosecurity and quarantine measures to minimize the disease occurrence.

Key words: Avian influenza, flocks, molecular, survey

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) of the subtype H5N1 is a contagious pathogen that causes severe respiratory disease with high mortality in poultry (Ali et al., 2015). Type A influenza belongs to the orthomyxoviridae virus family, is enveloped, and is pleomorphic with a size ranging from 80-120 nm (Spackman, 2008). According to Hemagglutinin (HA) and Neuraminidase (NA), there are 18 HA subtypes and 11 NA subtypes. Many different combinations of HA and NA proteins are possible (Tong et al., 2013). AI viruses are categorized as either Low Pathogenic (LP) or High Pathogenic (HP). HPAI viruses produce severe, systemic disease with high mortality in chickens and other gallinaceous poultry birds, but usually don't produce infection or possible mild disease in ducks. The newer H5 and the H7 HPAI viruses have shifted to increased virulence in chickens (Pantin-Jackwood and Swayne, 2009). HPAI H5N1 virus has been endemic in Egypt since 2006. The main symptoms were ecchymosis on the shanks and feet, cyanosis of the comb and wattles, subcutaneous edema of the head and neck for chickens, and nervous signs (torticollis) for ducks. Within 48-72 hours of the onset of illness, the average mortality rates were 22.8-30 % and 28.5-40 % in vaccinated chickens and non-vaccinated ducks respectively (Hagag et al., 2015). A newly emerged H5N8 influenza virus was isolated from green-winged teal in Egypt during December 2016. Multiple peculiar mutations were characterized in the Egyptian H5N8 viruses (Kandiel et al., 2017). Although some of the commercial vaccines protected chickens from mortality by H5N8, they failed to prevent chickens from shedding of virus. Accordingly, so the updating and reinforcing of H5N8 prevention and control strategies in Egypt is very important (Kandiel et al., 2018). Influenza viruses are inherently unstable, as these viruses lack a genetic proof-reading mechanism, small errors that occur when the virus copies itself go undetected and uncorrected. Specific mutations and evolution in influenza viruses cannot be predicted (WHO, 2006). Viruses in vaccinated poultry populations displayed higher mutation rates at the immunogenic epitopes, promoting viral escape and reducing vaccine efficiency (Abdelwhab et al., 2016). The aim of this study is epidemiological survey of AI in commercial

chicken flocks during 2016-2017 on El-Behera, El-Gharbia, Dimiatta governorates through Real Time Polymerase Chain Reaction (RT-PCR) and sequencing analysis to follow recent and current changes that occur on AI viruses.

MATERIAL AND METHODS

Chicken flocks

Twenty-eight broiler chicken flocks of different breeds and age ranged from (25-35 days) at El-Behera, El- Gharbia and Damietta governorates, Egypt during period from June 2016 to May 2017 showing high mortality rate with respiratory manifestations and cyanosis of comb and wattles and diarrhea, the course of disease ranged between 3-5 days. All flocks had history of non-vaccination against AI disease. Samples were taken from trachea, lung and liver of freshly slaughtered birds in each flock separately and transported to laboratory of poultry and fish disease department, faculty of veterinary medicine, Alexandria University, Egypt on ice box.

Virus isolation

The tissue was homogenized with three successive freezing and thawing forming tissue homogenate 10% with Phosphate Buffer Saline (PBS) and centrifuged at 3000 rpm for 15 min. Each sample was inoculated in five eggs with 0.1 ml of tissue homogenate per egg via allantoic sac route, two eggs inoculated with 0.1 ml PBS used as negative control. The fertile specific pathogen free Eggs (SPF ECEs) obtained from kom oshem, fayom, Egypt is used in present study. Inoculated eggs were incubated at 37°c for five days with daily candling for mortalities with discarding non- specific death at first 24 hours of inoculation (OIE, 2008). Dead embryos were examined for gross lesions and their allantoic fluids were harvested and tested by slide (HA) test (Charles, 1989) and the negative allantoic fluids were inoculated on SPF ECEs for two successive passages.

Real time-reverse transcription polymerase chain reaction (RRT-PCR)

Fourteen positive HA allantoic fluid samples were extracted by QIAamp viral RNA mini kits {cat.no .52904, (QIAGEN), Germany} as described by manufacturer manual of Qiagen RNA extraction kit. RRT-PCR was performed using primers of H5 (Lòndt et al., 2008), H9 (Ben Shabat et al., 2010), IB (Meir et al., 2010) and ND (Wise et al., 2004) as shown in table 1.

Sequencing and phylogenetic analysis

The positive samples for AI H5 by RT-PCR were subjected to nucleotide sequencing using primer according to Slomka et al. (2007) (Table 2) using 301bp on an Applied Bio systems 3130 automated DNA sequencer *(*ABI, 3130, USA*)* using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA) (cat-number 4336817) for performing gene sequencing using an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). QIA quick PCR product extraction kit (Qiagen Inc. Valencia CA), was used for purification of the PCR product on (1.5%) agarose gel directly. Using Centrisep (spin column, Cat number: CS-901) of 100 reactions according to the instruction of the manufacture for Purification of the sequence reaction. A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of Meg Align module of laser gene DNA star software Pairwise, which was designed by Thompson et al. (1994) to determine nucleotide and amino acid sequence similarities and relationships. Phylogenetic analysis was done using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

Ethical approval

Direct collection of tissues and organs from freshly slaughtered birds was carried out in strict accordance with the recommendations of Alexandria university, Egypt for the care and use of laboratory animals. Also, slaughtered chickens were humanly handled.

RESULTS

Investigated chicken flocks

Twenty-eight chicken farms suffered from clinical signs and post mortem lesions suspected to be of AI disease were investigated in this study. The diagnosis of AI depends on observation of clinical signs like cyanosis at comb, wattles and legs and sever sinusitis and severe respiratory signs and swelling in the head and post mortem pathological lesions like, tracheitis, petechial hemorrhage on proventriculus, congestion of pectoral and thigh muscles and all internal organs as liver, kidney, spleen, heart, brain and thymus in addition to sever pancreatitis with necrosis.

Virus isolation

All samples were isolated in fertilized SPF ECEs at age 10 days and the embryos mortality began after 48

hours post incubation. The dead embryos characterized by sever hemorrhage in all the body. By slide (HA), the positive samples were (3/8) from Dimiatta (4/9) from Gharbia and (7/11) from El-Behera (Table 4).

Real time-reverse transcription polymerase chain reaction

Concerning the results of RRT-PCR, (2/14) samples were positive to AI H5 (7/14) to NDV, (1/14) to H9 and (4/14) co-infected (2 samples had ND +AI H5 and others had (ND +AI H9) (Table 4).

Sequencing and phylogenetic analysis of H5N8 HPAI isolates results

The phylogenetic tree of HA gene was revealed that the two analyzed isolates (H5) were H5N8 HPAI Influenza Avirus{A/chicken/Egypt/Alex-1/2017(H5N8)} {A/chicken/Egypt/Alex-2/2017(H5N8)} and clustered together and belonged to clade 2.3.4.4 viruses which circulating in Russia and the two isolates were closely related to the first Egyptian isolates (EPIISL224580) which was named A/common coot/Egypt/CA285/2016 (EG-CA285) in Egypt which was isolated from El-fayoum (Figure 1). In addition, amino acid sequencing revealed that the arrangement (PLREKRRKR/GLF) was found in the two isolates and were compared with other H5N8 isolates and commercial AI vaccines on the gene bank and found that the two isolates located at one group with Russian strains (Table 3). These two isolates have sequence identity equal to 99%. But the similarities between two isolated samples and other H5N8 sample which recorded on gene bank ranged between (94.1-99.3%). The similarity between the two isolates and other isolate which isolated in Egypt (EPIISL) is 97.4 – 97.7 %, also percent of similarity between the two isolated samples and other vaccines for AI virus ranged from 80–91.7 % (Figure 2). These two isolates of H5N8 were submitted on gene bank under accession numbers MF182406 and MF182407.

Table 1. Oligonucleotide primers which used in RRT/PCR for identifying the samples oligonucleotide primers and probes used were supplied from Metabion (Germany) these analyses were done by national laboratory for veterinary quality control on poultry production, animal health research institute, Giza, Egypt at 2017

Primer/ probe sequence 5'-3'		References
Avian influenza (H5)	H5LH1(F)ACATATGACTAC CCACARTATTCAG H5RH1(R)AGACCAGCTAYCATGATTGC H5(PRO)[FAM]TCWACAGTGGCGAGT TCCCTAGCA[TAMRA]	Löndt et al., 2008
Avian influenza (H9)	H9(F)GGAAGAATTAATTATTATTGGTCGGTAC H9(R)GCCACCTTTTCAGTCTGACATT H9 (PRO)[FAM]AACCAGGCCAGACATTGCGAGTAAGATCC[BHQ]	BenShabat et al., 2010
Infectious Bronchitis (IB)	AIBV-(F)ATGCTCAACCTTGTCCCTAGCA AIBV-(R)TCAAACTGCGGATCATCACGT AIBV-(PRO)[FAM]TTGGAAGTAGAGTGACGCCCAAACTTCA [TAMRA]	Meir et al., 2010
New castle Disease Virus (NDV)	M+4100 (F)AGTGATGTGCTCGGACCTTC M-4220 (R)CCTGAGGAGAGGCATTTGCTA M+4169(PRO)[FAM]TTCTCTAGCAGTGGGACAGCCTGC [TAMRA]-3’	Wise et al., 2004

F: forward of the primer, R: reverse of the primer, PRO: probe of the primer

Table 2. Primers (for sequencing) of H5 isolates, primers were supplied from Metabion (Germany), these analyses were done by national laboratory for veterinary quality control on poultry production, animal health research institute, Giza, Egypt at 2017

Virus	Primer/ probe	Amplified product	Reference
H5	sequence5'-3'H5-kha-1(F)CCTCCAGARTATGCM TAY AAA ATT GTC H5-kha-3(R)TACCAACCGTCTACC ATKCCYTG	311 bp	Slomka *etal*., 2007

F: forward, R: reverse

Table 3. Amino acid sequence of H5N8 avian influenza virus subtype isolates these analyses were done by national laboratory for veterinary quality control on poultry production, animal health research institute, Giza, Egypt at 2017

Avianinfluenza virus	Amino acid sequence	Accession number
InfluenzaAvirus{A/chicken/Egypt/Alex1/2017(H5N8)}	PEYAYKIVKKGDSTIMKSEVEYGHCNTKCQTPVGAINSSMPFHNIHPLTIGECPKYVKSNKLVLATGLRNS***PLREKRRKRGLF***GAIAGFIEGGWQGMVDGW	MF182406
InfluenzaAvirus{A/chickenEgypt/Alex-2/2017(H5N8	PEYAYKIVKKGDSTIMKSEVEYGHCNTKCQTPVGAINSSMPFHNIHPLTIGECPKYVKSNKLVLATGLRNC***PLREKRRKRGLF***GAIAGFIEGGWQGMVDGWY	MF18240

F: forward, R: reverse

Table 4. History of investigated flocks, results of slide heamagglutination test and real time reverse transcriptase polymerase chain reaction, this investigation was done at 2016-2017

Number of flock	Age of birds /days	Breed of birds	Total number of flocks	Mortality %	Location	HA Slide test	RRT-PCR
1	26	Cobb	7000	8	El-Behera	+	NDV
2	29	Cobb	10000	9	EL-Behera	+	NDV
3	28	Avian48	15000	12	El-Behera	+	H9+NDV
4	33	Ross	5000	10	El-Behera	-	-
5	25	Avian48	6000	9	El-Behera	-	-
6	31	Ross	5000	9	El-Behera	-	-
7	35	Cobb	7000	8	El-Behera	-	-
8	34	Cobb	15000	20	Damietta	+	H5
9	33	Cobb	15000	8	Damietta	+	NDV
10	27	Cobb	20000	8	Damietta	+	NDV
11	29	Cobb	15000	9	Damietta	-	-
12	34	Avian48	5000	10	Damietta	-	-
13	26	Avian48	5000	8	Damietta	-	-
14	28	Ross	7000	8	Damietta	-	-
15	25	Cobb	5000	10	Damietta	-	-
16	33	Ross	8000	8	El-Gharbia	+	H9
17	31	Ross	20000	20	El-Gharbia	+	H5+NDV
18	34	Avian48	15000	20	El- Gharbia	+	H5+NDV
19	25	Cobb	10000	10	El- Gharbia	+	NDV
20	27	Cobb	6000	8	El-Gharbia	-	-
21	35	Avian48	10000	10	El-Gharbia	-	-
22	28	Ross	5000	19	El-Gharbia	-	-
23	28	Avian48	8000	10	El-Gharbia	-	-
24	35	Cobb	6000	8	El-Gharbia	-	-
25	27	Cobb	20000	15	El-Behera	+	H5
26	31	Avian48	5000	10	El-Behera	+	NDV
27	33	Cobb	6000	9	El-Behera	+	NDV
28	28	Cobb	10000	13	El-Behera	+	H9+ND

HA: hemagglutination test, RRT-PCR: real time reverse transcriptase polymerase chain reaction, H5: avian influenza virus H5, NDV: Newcastle Disease Virus, H9: avian influenza virus H9, +: positive, -: negative

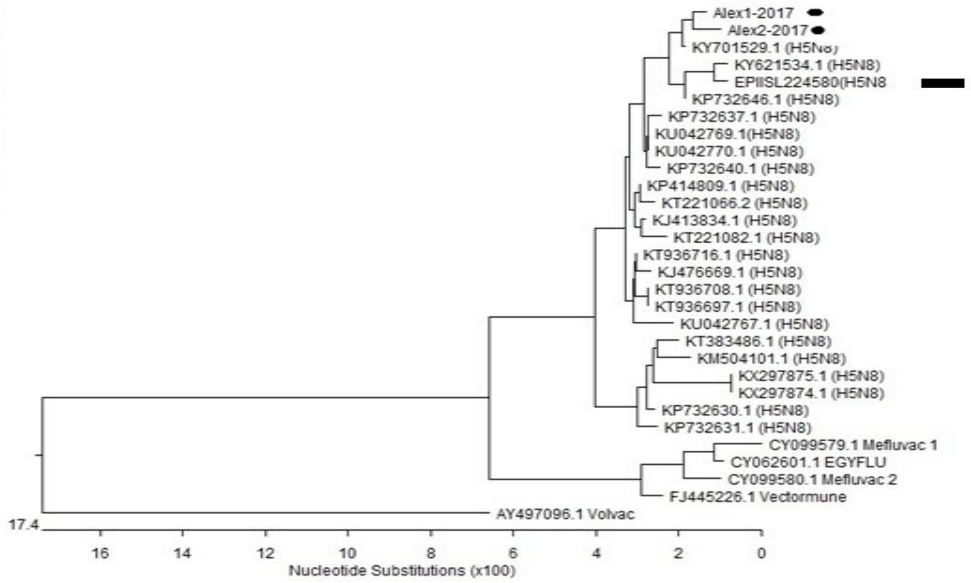

Figure 1. Phylogenetic tree for the two field isolates highly pathogenic H5N8 (●) and the first Egyptian isolate (■) (EPiiSL) with other H5N8 strain and commercial vaccines against avian influenza

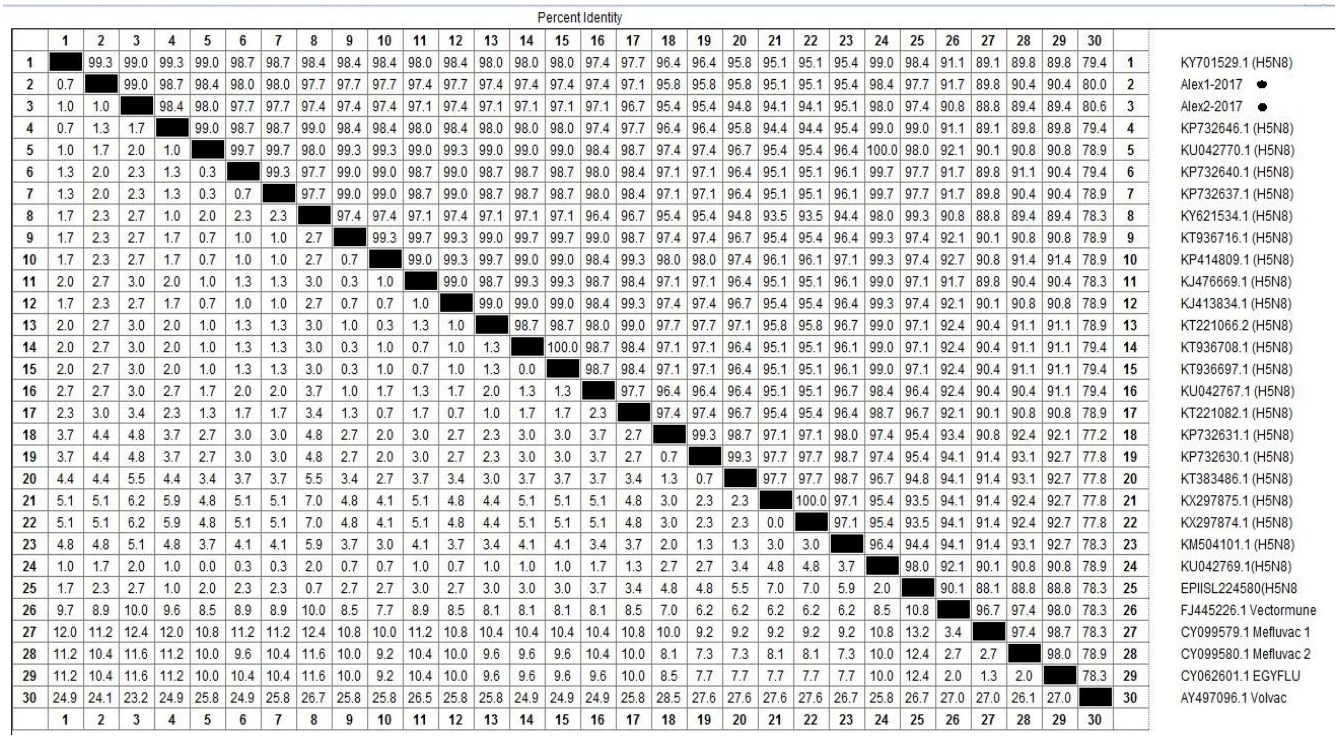

Figure 2. Identity and diversity of amino acid sequence of isolated tow field H5N8 (●) high pathogenic avian influenza compared with other H5N8 viruses and some commercial avian influenza vaccine

DISCUSSION

Highly pathogenic avian influenza A (H5N8) viruses of clade 2.3.4.4 spread into West Africa in late 2016 during the autumn bird migration. Genetic characterization of the complete genome of these viruses detected in wild and domestic birds in Cameroon on January 2017 demonstrated the occurrence of multiple virus introductions **(**Wade et al., 2018). The first report of highly pathogenic H5N8 avian influenza detection in Egypt, made it the third country in the Middle East that report the new H5 clade, which has also spread to several European countries after it was first identified in Russian migratory birds (Cidrap, 2016). The clinical signs and post mortem lesions recorded in this study supported by Abou Rawash et al. (2012); Horimoto and kawaoka (2001); Wasito et al. (2016); ElBakrey et al. (2013); Twabela et al. (2018). All investigated flocks were not vaccinated against avian influenza virus, and that may contribute to high mortality percent in the flocks. All samples were inoculated in SPF-ECEs as AI was completely adapted in (ECEs) via allantoic sac inoculation route , and lead to death of the embryo with hemorrhagic lesions (Brauer and chen, 2015; Salaheldin et al., 2017). Molecular identification of AI was carried out by using real time RT /PCR as it consider a sensitive method for accurate AI detection (Hanna et al., 2015; Bouwstra et al., 2015) and in present study, 4/28 samples were positive to H5 from which two samples were positive pure. Performing real time RT/PCR followed by sequencing and phylogenetic analysis is very important and valuable technique to know strains of AI (Salaheldin et al., 2018; Li et al., 2017; Bouwstra et al., 2015) Two PCR positive samples (pureH5)were sequenced. The partition of amino acids (PLREKRRKR/GLF) means that the two isolates are high pathogenic AI virus (Kandeil et al., 2017; Selim et al., 2017). EG-CA285 contain three amino acid assignment differences in the HA protein, namely R22K , E268G and D487Y which distinguished EG-CA285 from the recent HPAIV (H5N8) clade 2.3.4.4b strain isolated in Russia (A/great-crested-grebe/Uvs-Nuur-Lake/341/2016) and in the NA protein, they observed four substitution mutations (V8A, V31L, G126E,I407T) that distinguished the EG-CA285 from the subtype found in Russia (Selim et al., 2017). And Phylogenetic analysis explained that relation-ship between the first Egyptian H5N8 isolate (EPI ISL 224580), two H5N8isolates and other H5N8 isolates (Figure 1) so, the two isolates (Alex1, Alex2) are related to clade 2.3.4.4b.

A total 301 deduced amino acids sequences from a.a position 1 to 301 were analyzed**.** The two isolates had

some changes of amino acids like S71C, A176G, A211T and G225A in Alex1–Alex 2 respectively but this difference has very little effect on the percent of identity between the two isolates equal to 99%. Also identity percent between the two isolates and other commercial AI vaccines ranged from 80–91.7% which approve the little genetically relation-ship between the two isolates and commercial vaccines so chicken sera will be of little or no titer and this approved also by Kandeil et al. (2017) who stated that, chicken sera induced by commercial inactivated H5- vaccines showed no or very low reactivity with H5N8 viruses so, it should be depend on biosecurity in prevention programs against AI H5N8 and put all possible rules for protecting the poultry industry in Egypt. Egypt is consider the bridge between Europe, Asia and Africa where millions of migrating birds pass during their flights annually particularly in winter. Thus, Egypt is under huge pressure from migratory birds from the entire world and researchers must improve the criteria of deal with any spot of infection.

CONCLUSION

We concluded from present study many respiratory viral diseases threaten poultry industry in Egypt and new avian influenza H5N8 strain isolated from different area in Egypt in addition to high distribution of avian influenza H5N1, so this industry needs to more efforts by veterinary authorities to reduce these spreading in Egypt and protect the industry to keep away human from the zoonotic infections.

DECLARATIONS

Competing interests

The authors have no competing interests to declare.

Consent to publish

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

Author`s contributions.

All authors participated in making the design, performing the experiment, analyses of the data, and writing the paper.

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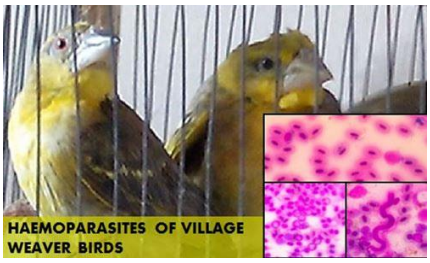

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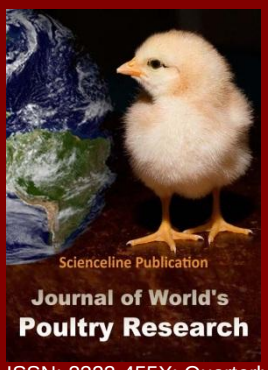

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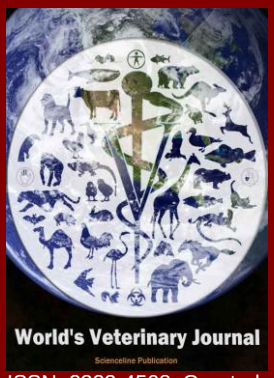

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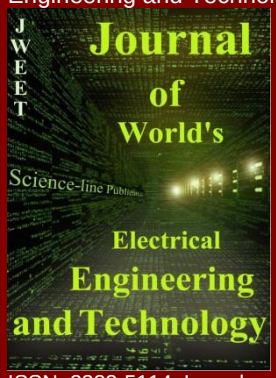

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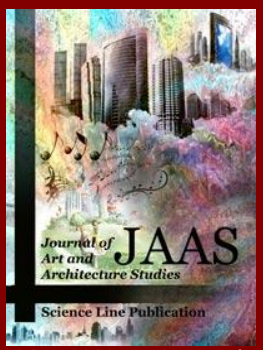

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