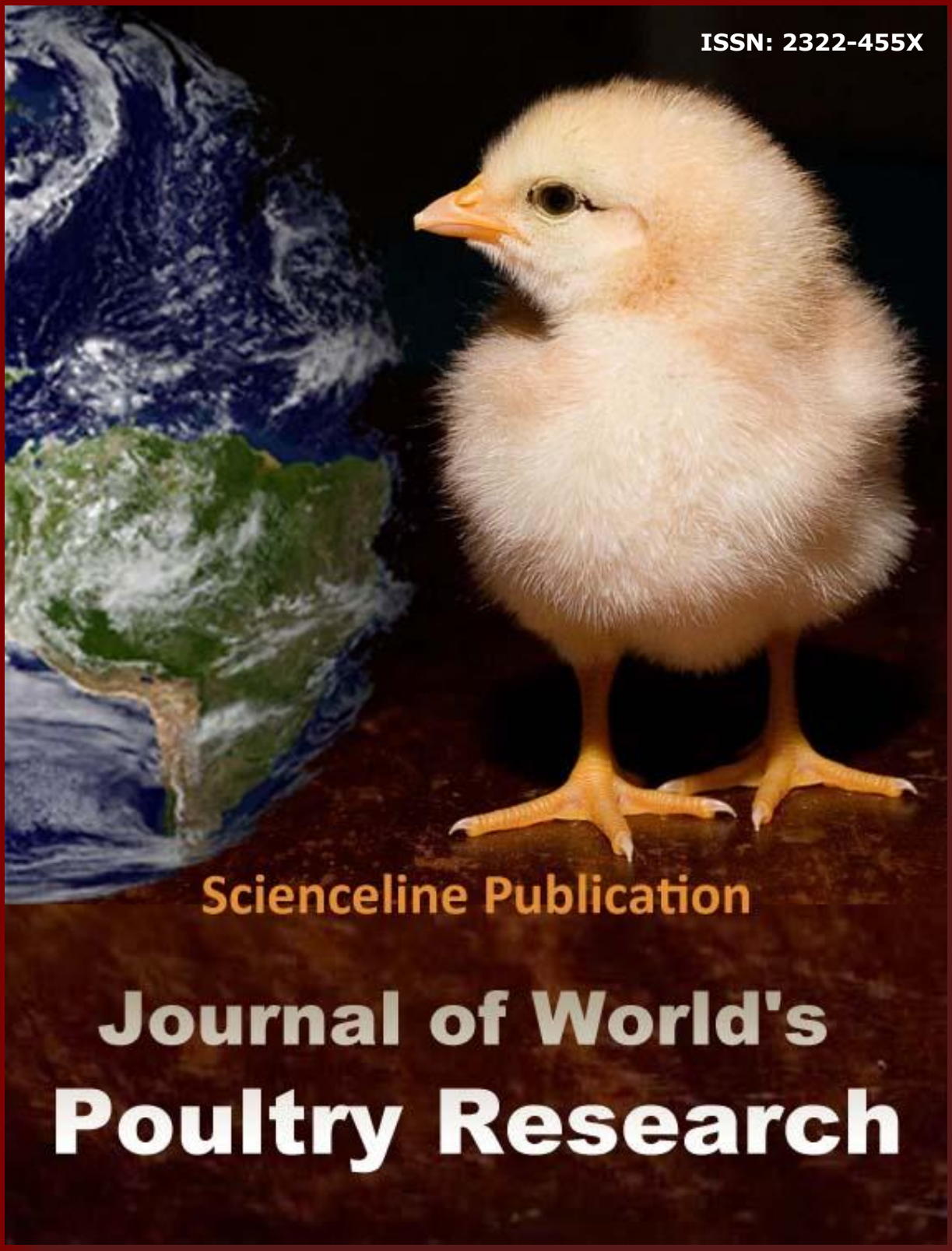
In the Name of God



An international peer-reviewed journal which publishes in electronic format

Volume 9, Issue 4, December 2019

Journal of World's Poultry Research

J. World Poult. Res. 9 (4): 160-174; December 25, 2019

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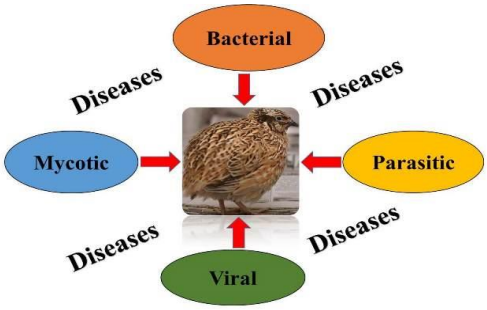
A Comprehensive Review on the Common Emerging Diseases in Quails.

Abd El-Ghany WA.
J. World Poult. Res. 9(4): 160-174, 2019; pii: S2322455X1900021-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.20



Abd El-Ghany WA (2019). **A Comprehensive Review on the Common Emerging Diseases in Quails.** *J. World Poult. Res.*, 9 (4): 160-174. http://jwpr.science-line.com

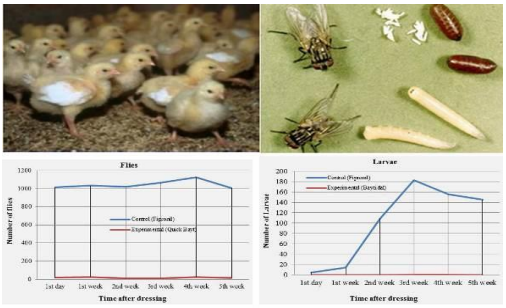
ABSTRACT: The poultry industry is considered an important sector that meets the great demand for protein sources all over the world. Now, quails are recognized as promising and important alternative species with many advantages over other poultry species. In many countries around the world, quail meat has achieved great popularity as a good source of protein and other important nutrients. However, there are some limitations and challenges to quails production. One of them is the susceptibility to some viral, bacterial, mycotic and parasitic diseases that can adversely affect quails. Many of the diseases that affect quails cause severe economic losses in quail industry due to a decrease in growth performance, poor feed conversion, reduction in hatchability, increased mortality and treatment costs. There are limited research and literature dealing with different disease and conditions affecting quails. Therefore, the aim of this work was to present a comprehensive review of the most important emerging diseases affecting quails worldwide.
Keywords: Bacteria, Virus, Mycosis, Myctoxicosis, Parasites, Quail

[Full text-PDF]

Research Paper

Systematic Program for Destroying of Flies' Population in Poultry Farm under Battery Cage Management in Russia.

Safiullin RT, Safiullin RR and Kachanova EO.
J. World Poult. Res. 9(4): 175-179, 2019; pii: S2322455X1900022-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.21



Safiullin RT, Safiullin RR and Kachanova EO (2020). **Systematic Program for Destroying of Flies' Population in Poultry Farm under Battery Cage Management in Russia.** *J. World Poult. Res.*, 9 (4): 175-179. http://jwpr.science-line.com

ABSTRACT: Favorable conditions for development, reproduction, and accumulation of large amounts of zoophilous flies in commercial poultry farms are caused by incomplete compliance with veterinary and sanitary rules for growing in cage facilities. The purpose of the study was to test a systematic insecticidal program for destroying flies' populations using adulticide and larvicide drugs in poultry farms under battery cage management. The number of imago flies in hen houses was dynamically evaluated using flypapers, six flypapers in each hen house, situated in different levels above the floor. Flypapers were removed and the number of stuck insects was counted. The number of larvae was evaluated in dynamics by specimen testing from the floor area 10x10 cm, with weight of 3-5 g. The Quick Bayt WG 10% was applied to destroy the imago of flies. Baycidal® WP 25% was used against larvae of flies. Complex insecticide program Quick Bayt WG 10% + Baycidal® WP 25% provided the opportunity to destroy flies, with a significant difference in intensefficacy, (98.3 % for adult flies and 99.8 % for larvae). Furthermore, this program had a positive impact on economic indicators of meat production of broilers. The present study demonstrated high preventive efficacy and economical efficacy of complex program against flies under battery cage broiler management.
Keywords: Adulticide, Economical Efficacy, Fly Larvae, Intensefficacy, Larvicide, Zoophilous Flies

[Full text-PDF]

Research Paper

Effects of *Moringa oleifera* and *Garcinia kola* with or without Grits on Haematological and Serum Biochemical Parameters of Broiler Chickens.

Adejola YA, Sobayo RA, Muhammad SB, Ayoola AA and Jinadu KB.
J. World Poult. Res. 9(4): 180-186, 2019; pii: S2322455X1900023-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.22

Adejola YA, Sobayo RA, Muhammad SB, Ayoola AA and Jinadu KB (2019). **Effects of *Moringa oleifera* and *Garcinia kola* with or without Grits on Haematological and Serum Biochemical Parameters of Broiler Chickens.** *J. World Poult. Res.*, 9 (4): 180-186. http://jwpr.science-line.com

ABSTRACT: The use of antibiotics as growth promoters in food animals has been banned due to the residual effects on final consumers which could lead to human health issues. The aim of the present study was to investigate the effects of two herbal feed additives with or without grits on hematological and serum biochemical

parameters of broiler chickens. One hundred and forty-four, one-day-old, Cobb 500 broiler chicks were randomly assigned into six treatments (24 birds per treatment) with three replicates (eight bird per replicate). Six dietary treatments were formulated with the inclusion of *Moringa oleifera* Leaf Meal (MOLM), *Garcinia kola* Seed Meal (GKSM) and grits. The experimental rations contained diet without MOLM, GKSM and grits which served as treatment 1 (control), diet with MOLM at 1000ppm (treatment 2), diet with GKSM at 1000ppm (treatment 3), diet with grits at 1000ppm (treatment 4), diet with MOLM at 1000ppm + grits at 1000ppm (treatment 5) and diet with GKSM at 1000ppm + grits at 1000ppm (treatment 6). Blood samples were collected on 28 and 56 days of age for hematological and biochemical analysis. Data were subjected to analysis of variance in a completely randomized design. At the starter phase, red blood cells (1.15 $\times 10^{12}$ L) and white blood cells were significantly lowest in birds of first treatment. The birds that received treatment 6, had the highest glucose (131.50 g/dl) and high-density lipoprotein level (58.50 mg/dl). At the finisher phase, the lowest white blood cell count (10.95 $\times 10^{9}$/L) and lymphocytes (60%) were recorded in treatment 6. Birds in treatment 3 indicated the lowest urea (2.05 mg/dl) and triglyceride (94.50 mg/dl). It can be concluded that diet supplemented with GKSM at 1000 ppm, increased high-density lipoprotein, and reduced triglyceride and low-density lipoprotein levels in serum of broiler chickens.

Keywords: Blood parameters, Feed additive, *Garcinia Kola*, Grit, *Moringa oleifera*

[Full text-PDF]

Research Paper

The Evaluation of Dietary Addition of Palm and Coconut Oils in Steaming Tomato (*Lycopersicon esculentum*) Waste Powder on Digestibility of Crude Fiber and Retention of Lycopene and Nitrogen in Broiler Chickens.

Handayani UF, Wizna, Suliansyah I, Rizal Y and Mahata ME (2019). **The Evaluation of Dietary Addition of Palm and Coconut Oils in Steaming Tomato (*Lycopersicon esculentum*) Waste Powder on Digestibility of Crude Fiber and Retention of Lycopene and Nitrogen in Broiler Chickens.** *J. World Poult. Res.*, 9 (4): 187-195. http://jwpr.science-line.com

Handayani UF, Wizna, Suliansyah I, Rizal Y and Mahata ME.
J. World Poult. Res. 9(4): 187-195, 2019; pii: S2322455X1900024-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.23

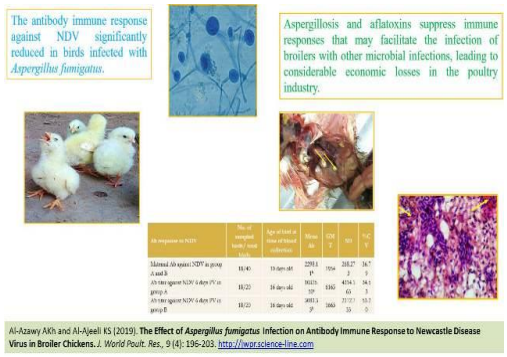
ABSTRACT: Lycopene is a powerful antioxidant present in tomatoes and other vegetables and fruits. Present research was carried out to evaluate lycopene and nitrogen retention and crude fiber (CF) digestibility of steaming tomatoes waste powder which was combined with oil. Tomatoes waste in this experiment were local fresh tomato rejected from tomato field around West Sumatera province, Indonesia. The experimental factors were included the type of oil (palm and coconut oils) and dosage of oils (0.25, 0.5, 0.75, 1, and 1.25 %), and each treatment was replicated three times. The results indicated there was an interaction between the type of oil and dosage of oil on lycopene retention, and CF digestibility, while the type of oil and dosage of oil affected lycopene retention significantly. The dosage of oil also influenced lycopene retention, nitrogen retention, CF digestibility significantly. The addition of coconut oil in steaming tomato waste powder increased lycopene and nitrogen retention, and CF digestibility higher than the addition of palm oil to steaming tomato waste powder in broiler chickens. The lycopene and nitrogen retention, and CF digestibility of steaming tomato waste powder added 0.5% coconut oil was the best level for lycopene and nitrogen retention, and CF digestibility in broiler chickens.

Keywords: Coconut oil, Crude fiber digestibility, Lycopene retention, Nitrogen retention, Palm oil, Tomatoes waste

[Full text-PDF]

Research Paper

The Effect of Aspergillus fumigatus Infection on Antibody Immune Response to Newcastle Disease Virus in Broiler Chickens.



Al-Azawy AKh and Al-Ajeeli KS (2019). **The Effect of *Aspergillus fumigatus* Infection on Antibody Immune Response to Newcastle Disease Virus in Broiler Chickens.** *J. World Poult. Res.*, 9 (4): 196-203. http://jwpr.science-line.com

Al-Azawy AKh and Al-Ajeeli KS.
J. World Poult. Res. 9(4): 196-203, 2019; pii: S2322455X1900025-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.24

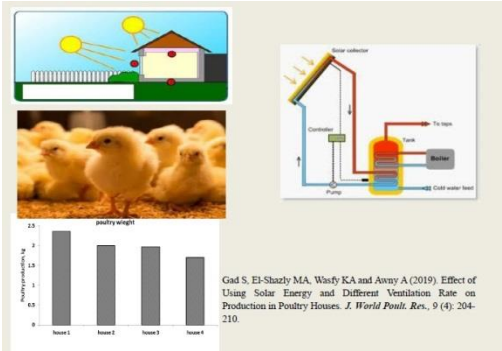
ABSTRACT: *Aspergillus fumigatus* infection might predispose birds to other respiratory infections with other pathogens such as Newcastle Disease Virus (NDV). This study aimed to investigate the incidence of *Aspergillus fumigatus* in commercial farms and its histopathological effects on respiratory organs and to evaluate the immunosuppressive effect of aspergillosis on NDV vaccinated birds. *Aspergillus fumigatus* was isolated from feedstuff and broilers in farms with respiratory manifestation. Twenty NDV-vaccinated broiler chickens of 10 days old were experimentally infected by feeding on feedstuff contaminated with *Aspergillus fumigatus*. Twenty vaccinated broilers but not fed the contaminated diet were used as the control group. Clinical signs, histopathological changes, NDV antibody levels in infected birds were recorded. Clinically, infected birds showed respiratory distress, dyspnea, gasping, ruffled feathers, green watery diarrhea, anorexia, lethargy, and unilateral drooping of wing. Histopathological changes were observed as disseminated granulomatous foci in the affected lungs, with caseous necrosis and leukocytes infiltration. The antibody immune response against NDV significantly reduced in infected birds compared with that of non-infected broilers. It is concluded, that *Aspergillus fumigatus* infection suppresses the immune responses and predisposes the broilers to other microbial infections, leading to considerable economic losses in the poultry industry.

Keywords: *Aspergillus fumigatus*, Immunosuppression, NDV vaccine

[Full text-PDF]

Research Paper

Effect of Using Solar Energy and Different Ventilation Rate on Production in Poultry Houses.



Gad S, El-Shazly MA, Wasfy KA and Awny A.
J. World Poult. Res. 9(4): 204-210, 2019; pii: S2322455X1900026-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.25

ABSTRACT: The main purpose of the present study was to find an alternative source for traditional energy to provide the energy requirements in the poultry industry. The present study was conducted in four poultry houses with different heating systems (solar and conventional) and ventilation rates located in El-Sharkia Governorate, Egypt, during June and July 2018. In this study, it was found that productivity increased by increasing the ventilation rate, where productivity reached 2.3 kg when using a solar heating system with a ventilation rate every two minutes. Productivity decreased in poultry houses with a conventional heating system and was 2 kg in ventilation rate every 2 minutes, and 1.8 kg in the ventilation rate every four minutes. The level of ammonia was also reduced with the ventilation rate every two minutes. Concentrations of ammonia ranged from 22 ppm at ventilation rate every two minutes to 28 ppm at the ventilation rate every four minutes. In addition, solar energy provided good levels of thermal requirements. It was demonstrated that solar energy as an alternative source to the conventional energy, is very efficient and can be applied on a large scale when combined with conventional electricity as a light source and within specified limits.
Keywords: Energy balance, Poultry production, Solar heating system, Ventilation

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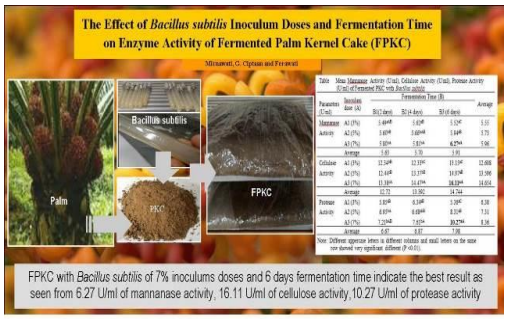
Research Paper

The Effect of Bacillus subtilis Inoculum Doses and Fermentation Time on Enzyme Activity of Fermented Palm Kernel Cake.



Mirnawati, Ciptaan G and Ferawati (2019). The Effect of *Bacillus subtilis* Inoculum Doses and Fermentation Time on Enzyme Activity of Fermented Palm Kernel Cake. *J. World Poult. Res.*, 9 (4): 211-216. http://jwpr.science-line.com

Mirnawati, Ciptaan G and Ferawati.
J. World Poult. Res. 9(4): 211-216, 2019; pii: S2322455X1900027-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.26

ABSTRACT: Palm kernel cake (PKC) was by-product of palm oil industry and it had potential to be one of the poultry ration ingredient However, its utilization for poultry was still limited because of the β-mannan in PKC. In order to increase PKC utilization in poultry ration, fermentation process was done to remodeled β mannan by using *Bacillus subtilis*. This research conducted a study on the effect of *Bacillus subtilis* inoculum dose and fermentation time to increase the enzyme activity of FPKC by using CRD with 3 × 3 factorial and 3 replications. Factor A was 3 doses of inoculum *Bacillus subtilis*: 3%, 5%, and 7%. Factor B was fermentation times which contained: (1) 2 days, (2) 4 days, and (3) 6 days. Parameters used were enzyme activity of mannanase, protease, and cellulase in FPKC. Significant interaction was seen between inoculum doses of *Bacillus subtilis* and fermentation time. There was also a significant interaction on each of the inoculums dose of *Bacillus subtilis* and fermentation time on all of the enzyme activity. This study concluded FPKC with *Bacillus subtilis* of 7% inoculums doses and 6 days fermentation time indicate the best result as seen from 24.27 U/ml of mannanase activity, 10.27 U/ml of protease activity, 17.13 U/ml of cellulose activity of fermented PKC.
Keywords: *Bacillus subtilis*, Enzyme activity, Fermentation time, Inoculum doses, Palm Kernel Cake
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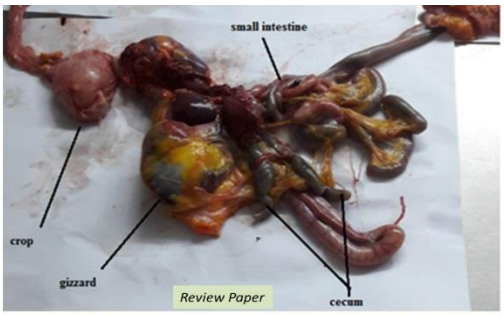
Review

Probiotics and Poultry Gut Microflora.



Tsega KT, Maina JK and Tesema NB (2019). **Probiotics and Poultry Gut Microflora.** *J. World Poult. Res.*, 9 (4): 217-223. http://jwpr.science-line.com

Tsega KT, Maina JK and Tesema NB.
J. World Poult. Res. 9(4): 217-223, 2019; pii: S2322455X1900028-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.27

ABSTRACT: Poultry production is presently the most effective animal production industry and provides an excellent source of protein production worldwide. The poultry gastrointestinal microbiota includes commensal, mutualistic and pathogenic microbes. The relationship between host and gut microbiota can affect the balance of mutualism and pathogenicity. The imbalanced gut microflora caused by the incidence of disease, hygiene conditions, diet, management practices, and environmental stress affects the survival and productivity of chicken. Maintenance of the gut microbial composition is possible through the regulation of the gastrointestinal microbiota by suppressing the growth of pathogens. For many years, antibiotic growth promoters have been used to manage these problems. Nowadays, because of the emergence of antibiotic-resistant bacteria, other alternatives are being sought. Supplementation of probiotics as feed additives is considered to enhance chicken productivitity and to protect the gut from pathogen colonization and help

to tolerate environmental stress. The goal of the present article was to review the poultry gastrointestinal microflora and probiotics role in the health and growth of poultry. In addition, this article focused on probiotic microorganisms and their potential characteristics.
Key words: Gastrointestinal microbiota, Poultry, Probiotics
[Full text-PDF]

Review

History and Current Situation of Commercial Ostrich Farming in Mexico.

Islas-Moreno A and Rendón-Medel R.
J. World Poult. Res. 9(4): 224-232, 2019; pii: S2322455X1900029-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.28

ABSTRACT: As in many other countries, in Mexico, the ostrich aroused the interest of public and private entities for its broad productive qualities and quality of its products. The objective of the present study was to describe the history of ostrich introduction in Mexico as a kind of commercial interest, from the arrival of the first birds to the current farms. In 1988 the first farm was established, then a series of farms of significant size were appearing, all of them focused their business on the sale of breeding stock, a business that was profitable during the heyday of the specie in the country (1998-2008). The main client was the government that acquired ostriches to distribute them among a large number of new farmers. When the introduction into the activity of government and private individuals was no longer attractive, the prices of the breeders fell and the sector collapsed because the farms were inefficient and the infrastructure and promotion sufficient to position the ostrich products were not produced on the national or export market. In 2016 it was known that about 30 farms remained in the activity, of which 20 were located and provided information for this study. The farms that remained in the activity continued with significant difficulties in terms of their productivity, however, they had managed to mitigate part of the problem by sharing production practices among themselves and going to their counterparts abroad through digital media. On the commercial side, they had managed to develop standardized products using maquiladora companies, and placed them in niche markets that paid for higher prices than those that are paid for conventional substitutes. In the case of ostrich, in Mexico and many other countries, the sector failed because the market demand response was overestimated and the farmers ventured into the activity without adequate knowledge bases, infrastructure, and institutional support. These findings could be referred to many other species of nascent interest.
Keywords: Emerging sectors, Exotic poultry, Niche market, Specialty livestock, Organization, Ostrich meat

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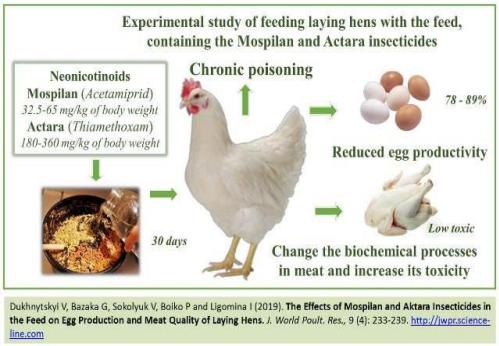
Research Paper

The Effects of Mospilan and Aktara Insecticides in the Feed on Egg Production and Meat Quality of Laying Hens.

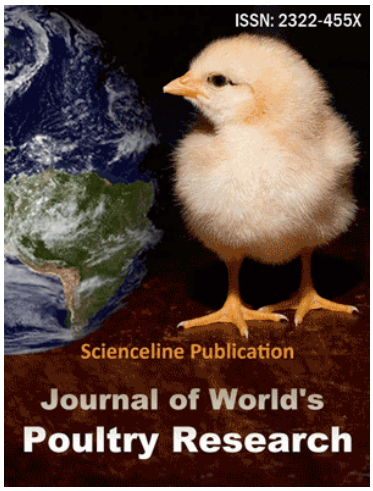


Dukhnytskyi V, Bazaka G, Sokolyuk V, Boiko P and Ligomina I.
J. World Poult. Res. 9(4): 233-239, 2019; pii: S2322455X1900030-9
DOI: https://dx.doi.org/10.36380/jwpr.2019.29

ABSTRACT: The current study was aimed to investigate the effects of feeding Mospilan and Actara insecticides on egg production performance and meat quality of laying hens. Experimental research was conducted in the laboratory of the Department of Pharmacology and Toxicology of the National University of Life and Environmental Sciences of Ukraine in 2015. The experiments were performed on five groups each consisting of seven chickens. The age of the chickens at the beginning of the experiment was 150 days. The birds were fed the granulated compound feed. In M1 and M2 groups, Mospilan at doses of 65 mg/kg and 32.5 mg/kg of body weight were added to the feed, respectively. In A1 and A2 groups, Actara at doses of 360 mg/kg and 180 mg/kg of body weight were added to the feed, respectively. Chickens of the control group were fed without the addition of insecticides to the feed. The feeding period lasted 30 days and finally, egg production performance, meat quality, and gross pathological changes were evaluated. Egg production rate in M1 and M2 groups in comparison to the control group decreased by 78.4 and 29.7%, respectively. Egg production rate in A1 and A2 groups reduced by 89.2% and 48.7% compared to the control group, respectively. Chickens in groups of receiving insecticides had pale skin and enlarged heart, also showed spot hemorrhages in mucous membranes of the glandular stomach and intestine, color heterogeneity of the lungs, and the liver was dark cherry in color with hemorrhage. In addition, the relative weights of internal organs decreased by 23-36% in experimental groups. In the experimental groups, the pH of meat decreased at day 4 post-slaughter, and the meat broth with the addition of 5% copper sulfate solution was slightly cloudy with flakes. The meat of birds from the experimental groups was low toxic. Extracts from chicken meat of the experimental groups caused pathological changes, inhibition of movements and death of 13-16% of Tetrahymena pyriformis infusoria. This study demonstrated that the presence of Mospilan and Aktara in feed reduced the egg production rate, caused chronic poisoning, changed biochemical processes in chicken meat and increased its toxicity.
Keywords: Chicken meat quality, Egg productivity, Insecticides Mospilan and Actara, Laying hens, Neonicotinoids.
[Full text-PDF]

Archive

Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

Current Issue: 2019, Vol: 9, Issue: 4 (December 25)

Publisher: SCIENCELINE

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

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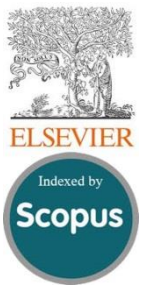

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 160-174, December 25, 2019

Review Paper, PII: S2322455X1900021-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.20

A Comprehensive Review on the Common Emerging Diseases in Quails

Wafaa A. Abd El-Ghany

Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 1211, Giza, Egypt

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Received: 06 Oct. 2019
Accepted: 13 Nov. 2019

ABSTRACT

The poultry industry is considered an important sector that meets the great demand for protein sources all over the world. Now, quails are recognized as promising and important alternative species with many advantages over other poultry species. In many countries around the world, quail meat has achieved great popularity as a good source of protein and other important nutrients. However, there are some limitations and challenges to quails production. One of them is the susceptibility to some viral, bacterial, mycotic and parasitic diseases that can adversely affect quails. Many of the diseases that affect quails cause severe economic losses in quail industry due to a decrease in growth performance, poor feed conversion, reduction in hatchability, increased mortality and treatment costs. There are limited research and literature dealing with different disease and conditions affecting quails. Therefore, the aim of this work was to present a comprehensive review of the most important emerging diseases affecting quails worldwide.

Key words: Bacteria, Virus, Mycosis, Myctoxicosis, Parasites, Quail

INTRODUCTION

The term quail refers to medium-sized birds belonging to various genera of family Phasianidae. There are two important species, the Japanese quails (*Coturnix japonica*) and Bobwhites quails (*Colinus virginianus*) which considered domestic birds since 14th century (Arya et al., 2018).

In recent years, the quail industry has created a huge impact and has been widely distributed in several countries around the world (Murakami, 1991; Redoy et al., 2017). Quails production is more profitable because it requires less investment to start and provides quick returns with a higher cost-benefit ratio. Quail farming is gaining popularity because quails are easy to manage and small in size thus they can be raised within small floor space (Ruskin, 1991; Edris et al., 2004). Moreover, less feed requirements, rapid growth rate, palatable meat, high egg production, high nutritional value of meat and egg, early sexual maturity, short generation interval (3-4 generations per year) and short incubation period are other benefits of quail rearing (Hassan et al., 2017; Yambayamba and Chileshe, 2019).

The advancement in quail production is being hampered by some management factors, infectious and non-infectious diseases (Barnes and Gross, 1997). Infectious diseases are common in quails reared under intensive production system (Paulillo, 1989). As quails are related to poultry, several diseases affecting quails are similar to those in chickens and turkeys (Myint and Carter, 1988).

Accordingly, this review highlighted the most important viral, bacterial, mycotic and parasitic diseases as well as mycotoxicosis affecting quails species worldwide.

Viral diseases

Adenovirus

In Bobwhite quails, adenovirus induced acute respiratory contagious infection called quail bronchitis (Olsen, 1950; DuBose et al., 1958). This disease is more severe in young quails less than 3-week-old and leads to 100% morbidity and 50% mortality (Jack and Reed, 1990). Quail bronchitis virus was recently isolated from 5-day to 8-week old Bobwhite quails raised in Minnesota, USA.

The birds showed respiratory manifestations and high mortality, also histopathological findings included mucus in trachea, congested lungs, caseous airsacculitis, enlarged spleen, urates on internal organs, and necrotic foci on the liver (Singh et al., 2016).

Inclusion Body Hepatitis (IBH), caused by avian adenovirus-1, occurs in Bobwhite quails less than 3-week-old (Jack et al., 1987). However, the outbreak of IBH has been described in adult Japanese quail (Grewal et al., 1994). In a study by Singh et al. (1995), Japanese quails more than 4-week-old inoculated intraperitoneally with IBH adenovirus and showed congested pneumonic lungs, swollen mottled liver and necrotic kidney with intranuclear inclusion bodies. In addition, quail strains of IBH virus were pathogenic for broiler chickens after experimental inoculation.

Egg Drop Syndrome-76 (EDS-76), caused by avian adenovirus-3, was isolated from natural outbreaks in Japanese quail and the virus was serologically indistinguishable from that of chicken (Dash and Pradhan, 1990; Kataria et al., 1991; Dash and Pradhan, 1992). In a large study, the EDS-76 virus caused histopathological changes in the genitalia and spleen, decreased egg quantity and egg quality, increased virus antibody titers and total protein levels in laying Japanese quails (Mohapatra et al., 2014).

Experimental infection of 12-day-old quails with Chicken Embryo Lethal Orphan (CELO) virus and Avian Adeno-Associated Virus (AAAV) significantly increased CELO virus-produced mortality while double infections with high doses of AAAV induced a delay in mortality (Bagshaw et al., 1980).

The adenoviral inclusions were detected in the intestinal epithelia and glandular epithelium of gizzard and conjunctiva in less than 3-week-old quails with depression, ruffled feathers, diarrhea and high mortality (Tsai et al., 1998). Moreover, adenovirus inclusion bodies were observed in the proventriculus of Bobwhite quails and chickens. In wild Bobwhite quails with hepatic inclusion bodies, adenovirus serotype TR-59 was identified in their caeca (King et al., 1981).

Good management practice and biosecurity measures are very important to prevent adenovirus infections. There is no treatment for adenovirus infection. Water treatment with minerals and vitamins is critical to increase quail immunity (Singh et al., 2016).

Avian influenza

Avian Influenza Virus (AIV) was first recorded in Italy in Japanese quail under 3 months of age with respiratory manifestations and high mortalities (Nardelli et al., 1970). In several countries, many subtypes of AIV outbreaks have been detected in quail flocks (Guo et al., 2000; Wee et al., 2006; Lee et al., 2008; Yee et al., 2011; Arya et al., 2018). Japanese quail is considered a vehicle for adaptation of AIV strains in wild birds which is a means to generate new variant strains able to cross species barrier and infect different poultry species and possibly human (Perez et al., 2003; Wan and Perez, 2006; Wang et al., 2008). Chicken and quails were found to be highly susceptible to infection Highly Pathogenic (HP) AIV H5N1; while ducks have higher resistance and served as carriers (Hulse-Post et al., 2005; Tiensin et al., 2005). Moreover, HPAIV H5N1 strains isolated from geese were capable of causing disease in quails, with a longer period of virus shedding than that in chickens (Webster et al., 2002; Jeong et al., 2009; Saito et al., 2009). However, it was found that Japanese quails are resistant to HPAIV H5N3, which is pathogenic for turkeys, thus they could transmit this lethal virus to chickens (Tashiro et al., 1987). A study conducted in Korea revealed that chickens, ducks, and quails experimentally infected with HPAIV H5N1 had various symptoms, mortality, viral titers, and virus shedding. The mentioned study suggested that duck and quail farms should be regularly monitored to prevent virus transmission to another host (Jeong et al., 2009). European quails experimentally infected with HPAIV H7N1 and H5N1 showed severe nervous manifestations with histopathological changes and mortality rates of 67% and 92% in H7N1 and H5N1 challenged birds, respectively. While birds challenged with Low Pathogenic (LP) AIV H7N2 showed no clinical or pathological conditions. However, viral shedding and transmission to naive quail were observed for all types of AIV and drinking water and feathers were possible routes of HPAIV transmission (Bertran et al., 2013). In Egypt, a study on quails detected maternal antibodies against AIV and also evaluated immune responses to inactivated AI vaccines containing H5N1 and H5N2 viruses. The results revealed high to moderate levels of maternal immunity on the first and fifth days of age and low levels on the seventh day. In addition, vaccination at 8-day-old quails induced satisfactory titers at third week of post-vaccination, while the highest titers were detected at fourth and fifth weeks after vaccination (Saad et al., 2010). Moreover, H5N8 strain was detected in only one domestic Egyptian quail farm and it was also isolated from 2 out of 3 wild quail samples (Shehata et al., 2019). Outbreaks of AIV H9N2 revealed stable lineages in chickens and other poultry species such as quails (Naeem et al., 1999). In Hong Kong, 16% of quails in markets

were found to be positive for H9N2 viruses (Guan et al., 2000). The amino acid patterns of hemagglutinin of AIV H9 in quails were found to be intermediate between those in duck and in chicken, which explains the susceptibility of quail to duck AIV H9 (Perez et al., 2003). AIV could be detected using indirect immunofluorescent assay in the muscles and internal organs of quails, chickens, and ducks (Antarasena et al., 2006). Prevention of avian influenza in quails mainly occurs through vaccination combined with implementing biosecurity measures by thorough cleaning and disinfecting and restricting the personal movements on the farm. In a study conducted in China, the protective efficacy of inactivated H5N1 (clade 1) influenza vaccine (NIBRG-14) before challenge with heterologous A/Swan/Nagybaracska/01/06 (H5N1 clade 2.2) strain was tested in quails; the results revealed protection of challenged birds and absence of the virus in cloacal swabs, but immunized birds had low antibody titers (Sarkadi et al., 2013). In Indonesia, it was found that vaccination of brown quails with inactivated bivalent H5N1 clades 2.1.3 and 2.3.2 at ages 24 and 45 days induced significant protection, although the virus shedding continued 7 days post-vaccination (Indriani and Dharmayanti, 2016).

Newcastle disease

The pathogenicity of Newcastle Disease Virus (NDV) in quails depends on the virus strain, dose, and route of administration (Oladele et al., 2008). Quails are considered a susceptible host for NDV and have a non-negligible role in transmission of the virus to chickens. Therefore, quails should be vaccinated against the virus to protect them and prevent transmission to chickens (Sharawi et al., 2015). In Northern India, an outbreak of ND was recorded in Japanese quail aged 22-week-old with central nervous system manifestations, 30% mortality, and 20% morbidity. (Gowthaman et al., 2013). In Iran, loss of appetite, decreased egg production, diarrhea, and nervous symptoms were recorded in quails infected with NDV (Shoushtari et al., 2007). There are several reports on outbreaks of ND in Japanese quail flocks (Kaleta and Baldauf, 1988; Chandrasekaran and Aziz, 1989; Islam et al., 1994). Islam et al. (2016) found that NDV had the highest prevalence rate (11.35%) among the isolated viral diseases (25.21%) from 476 quails in Bangladesh. The experimental infection of quails with NDV revealed that oculo-nasal inoculation of 17-week-old Japanese quails with a velogenic NDV strain resulted in no morbidity or mortality, although broilers chickens that were in contact with infected birds showed clinical signs of ND with 100% mortality. These findings suggest that quails can be NDV carriers (Lima et al., 2004). Inoculation of velogenic strain of NDV in 3-6-week old Japanese quail induced different mortality rates according to route of inoculation, as follows: 13% (Usman et al., 2008) and 100% (Mohamed and Abdel Hafez, 2016) using oculo-nasal route, 25% using intracoelomic route (El-Tarabili et al., 2009), 3% (Oladele et al., 2008) and 40% (Sharawi et al., 2015) using intramuscular. Recently, in a study by Susta et al. (2018), 2-week-old Japanese quails were inoculated through the oculo-nasal route with 4 virulent NDV strains of different genotypes. The results indicated mild to moderate disease with mortality rate ranged from 28% to less than 10%, neurological signs with suppurative encephalitis. In addition, the virus replication was moderate in inoculated birds, but minimal in contact birds. Moreover, inoculated birds with NDV strains originated from quails showed high virus shedding, while high virus transmission occurred in birds inoculated with virus originated from chicken. Mazlan et al. (2017) proved susceptibility of Japanese quail to experimental infection with genotype VII NDV based on the development of specific clinical signs, detection of the virus antigen in the tissues and increase in the titers of haemagglutinating antibodies. The immune response to NDV vaccines in different lines of Japanese quail was studied and the results showed that double vaccination at 4 and 6 weeks of age with inactivated vaccine induced high antibody level in the high line breed that was 24% greater than control one, but in the low line, the antibody level was 37% less than that in the control (Takahashi et al., 1984). Vaccination using both living vaccines like La Sota or Hitchner B1 and inactivated vaccines is very important for efficient eradication of NDV in quails (Lima et al., 2004 and Paulillo et al., 2009).

Poxvirus

Although avian pox has been reported in a wide variety of domestic and wild birds (Bolte et al., 1999), the infection is not common in quails (Rinaldi et al., 1972; Crawford et al., 1979; Davidson et al., 1980; Poonacha and Wilson, 1981). Diphtheritic form of avian pox virus in the respiratory tract was found to induce significant mortality (Tripathy and Reed, 1997). Quail pox virus is a distinct species of the genus *Avipoxviridae*, and the virus had no immunologic relationship to pigeon and fowl poxviruses. Moreover, in areas where poultry is reared in close proximity to quails, cross-infection is possible (Winterfield and Reed, 1985; Ghildyal et al., 1989). Dry pox in quails causes lesions with a gray to yellow or dark brown discoloration and single or multiple nodules with

crusts in variable sizes on the comb, eyelids, and the other poorly feathered areas of the body (Singh et al., 1992; Gülbahar et al., 2005). Vaccination of quails is necessary for endemic areas. Quail pox can affect chickens. Fowlpox and pigeon pox vaccines could be considered good vaccines to control poxvirus infection in quails (Promkuntod et al., 2003).

Tumors

There are few reports on naturally occurring lymphoproliferative disease or Marek's Disease (MD) in Japanese quails. The MD Virus (MDV) is a causative agent of spontaneous tumor disease in quails (Pradhan et al., 1985; Adedeji et al., 2019) and can be transmitted to chickens by contact exposure (Kenzy and Cho, 1969). A study found a positive association between the incidence of lymphomatous changes and the presence of MDV-specific antigen on a quail flock (Kobayushi and Mikami, 1986). It has been reported that JM strain of MDV could be isolated by cell culture from quails at 7-8 days post-inoculation. (Khare et al., 1975), however, the same strain was not recovered from quail by direct culture of kidney tissue (Mikami et al., 1975). Quails inoculated with HPRS-16 strain of MDV showed lower viremia than that of chickens inoculated with the same strain (Powell and Rennie, 1984). Quails could be experimentally infected with MDV of chicken origin (Dutton et al., 1973; Fujimoto et al., 1975). The MDV was detected in natural lymphoproliferative outbreaks in eight flocks of Japanese quails. (Imai et al., 1990). Avian Leukosis Virus (ALV) could induce tumors in Japanese quails (Wight, 1963) and quails with lymphoproliferative disease had antibodies against ALV subgroup A (Schat et al., 1976). Recently, it was observed that intraperitoneal inoculation of quails with ALV subgroup A induced transient viremia, intermittent cloacal shedding and mild lesions in infected quails (Zhang et al., 2019). Quails are susceptible to experimental infection with reticuloendotheliosis virus (Theilen et al., 1966). A malignant tumor resembling reticuloendotheliosis was reported under natural conditions in quails (Carlson et al., 1974; Schat et al., 1976). In Japanese quail, avian myeloblastosis virus was able to produce a wide spectrum of neoplasms similar to that observed in chickens; however, contrary to what is observed in chickens, acute myeloblastic leukemia was not found in Japanese quail (Moscovici and Macintyre, 1966). Genetic selection of tumors-resistant breeds is very important to prevent tumor development in quails.

Bacterial diseases

Salmonellosis

Today, avian salmonellosis is still a major problem facing quail production and needs to be solved. Many reports demonstrated the isolation of different *Salmonella* spp. from quails (Erdogrul et al., 2002; Takata et al., 2003; Aarestrup et al., 2005). Sander et al. (2001) and Bacci et al. (2012) isolated *Salmonella enterica* in quail's carcasses. The frequency of salmonellosis in young quails of two flocks in Bangladesh was 6.73% and 11.97% (Islam et al., 2003, 2016). Al-Nakhli (2005) isolated different types of *Salmonella* spp. causing paratyphoid infection among Japanese quails in Saudi Arabia. In Brazilian quail flocks, Freitas et al. (2013) identified *S. enterica* subspecies Enterica; *S. Corvalis*, *S. Give*, *S. Lexington*, *S. Minnesota*, *S. Schwarzengrund*, *S. Rissen*, and *S. Typhimurium* from meconium samples of one-day-old quail chicks. In addition, *Salmonella* spp. were isolated from cloacal swabs of Kelantan quails in Malaysia (Palanisamy and Bamaiyi, 2015). Udhayavel et al. (2016) confirmed the identification of *S. enterica* from heart blood swabs, liver and spleen samples collected from 8-day-old Japanese quails in India. Recently, in Nigeria, a total of 19 out of 200 quail's eggs swabs were identified as *Salmonella* spp. (Mera et al., 2017). Moreover, it was found that 10 out of 75 (13.33%) quail samples from three farms in Bangladesh were positive for *Salmonella* spp. of which seven isolates were motile *Salmonella* (Jahan et al., 2018). Barde (2014) demonstrated that *S. Gallinarum* in Japanese quails causes septicemic disease with distribution of the organism in major organs, greenish-yellow diarrhea, high mortality, marked drop in egg production and congestion with enlargement of internal organs, which is similar to that in chickens. Hamed and Hassan (2013) proved that water supplementation with acetic acids, organic acids mixture, and hydrochloric acid reduced *S.* Enteritidis colonization in the gut and internal organs as well as inducing high protection from morbidity and mortality in quails. Nowadays, *in-ovo* inoculation of antibiotics is considered a new trend to prevent the possibility of bacterial pathogen transmission through eggs (Tavakkoli and Gooshki, 2014). In a study by Jahan et al. (2018), the *in-vitro* antibiotic sensitivity test of *Salmonella* strains isolated from quails showed that 100% of strains were resistant to erythromycin and tetracycline, but were sensitive to ciprofloxacin and imipenem, 90% of strains were resistant to colistin sulfate and 80% were sensitive to neomycin. Also, all *Salmonella* isolates showed multidrug resistance.

Colibacillosis

Septicemic colibacillosis caused by *Escherichia coli* (*E. coli*) is an infectious avian disease that has been commonly reported in chicken, turkeys, ducks, and quails (Da Silva et al., 1989). Infection with *E. coli* was associated with several diseases including yolk sac infection, septicemia, airsacculitis, peritonitis, polyserositis, omphalitis, cellulitis, coligranuloma and enteritis (Barnes and Gross, 1997; Dho-Moulin and Fairbrother, 1999). Arenas et al. (1999) isolated *E. coli* serogroup O165 from the internal organs of 4-6 day old Japanese quails with 90% mortality rate. The *E. coli* infection caused hepatitis and pericarditis in Japanese quails at 21 days and 11 months of age (Ito et al., 1990), and coligranulomatosis in common quails at the ages of 8-12 months (Da Silva et al., 1989). In Bobwhite quails, *E. coli* was isolated from specimens of liver, spleen, and intestine (Radi, 2004). Roy et al. (2006) isolated *E. coli* serogroups such as O4, O9, O38, O42, and O88 from diseased Japanese quail, dead-in-shell embryos, fluff samples, footbath and drinking water samples in a hatchery. The *E. coli* isolates cultured from infected Japanese quails belonged mainly to serogroup O9 (54.5%) and the same serotype was also predominant in the hatchery environment (Roy et al., 2006). The capability of *E. coli* serogroup O2 to produce dose-dependent cellulitis, pericarditis, perihepatitis, and septicemia in quails was recorded (Burns et al., 2003; Nain and Smits, 2011). In Iraq, 37 out of 203 (18.2%) bacterial isolates obtained from liver, lung, gizzard, and intestine of 30 healthy quails identified as *E. coli* (Hamad et al., 2012). In India, 32 out of 154 *E. coli* isolates (20.77%) were detected in different organs of quail birds (Manickam et al., 2017). However, in Nigeria, 21 out of 200 eggs swabs identified as *E. coli*, of which 11/21 (52.4%) were from eggshell swabs and 13/21 (61.9%) were from internal egg contents (Mera et al., 2017). In Bangladesh, the prevalence rate of colibacillosis in quails was 15.34%, which was the highest rate among bacterial disease (Islam et al., 2016). The isolation rate of *E. coli* among Japanese quail of Sylhet and Narsingdi region in Bangladesh was 5.17% and 5.7%, respectively (Islam et al., 2003; Uddin et al., 2010). Antibiotic sensitivity tests should be used to select the suitable specific antimicrobials for the treatment of specific *E. coli* serotypes.

Clostridial infection

Clostridial enteritis is a common problem in avian species (Ficken and Wage, 1997; Prescott, 2016). Ulcerative enteritis caused by *Clostridium colinum* (*C. colinum*) was recorded as an epidemic disease in Bobwhite quails (Berkhoff, 1975 and Cooper et al., 2013). Some outbreaks of highly contagious ulcerative enteritis have been described (Berkhoff and Kanitz, 1976; Berkhoff, 1985). Radi (2004) isolated *C. perfringens* from the intestine of Bobwhite quail with a history of anorexia, diarrhea, dehydration, weight loss, and acute death. In addition, ulceration and perforation of intestine, peritonitis, and multifocal necrotizing hepatitis were observed in histopathological examination. The detection limit of *C. colinum* in quails was $1.6X10^4$ colony forming units/g feces (Bano et al., 2008). Ulcerative enteritis-like disease due to *C. perfringens* type A was attributed as the cause of mortality in 10 to 16-week-old Bobwhite quails (Shivaprasad et al., 2008). Also, *C. sordellii* was associated with ulcerative enteritis in quails (Crespo et al., 2013). Penicillin-streptomycin was the most effective prophylactic and streptomycin was the most effective therapeutic agent for ulcerative enteritis in Bobwhite quail (Brown et al., 1970). Five days water treatment with tylosin was effective in controlling ulcerative enteritis in Bobwhite quails (Jones et al., 1976). Beltran-Alcrudo et al. (2008) described an outbreak of ulcerative enteritis caused by *C. colinum*, *C. perfringens* and *Eimeria* spp. in Bobwhite quail farm and found that combined treatment with an anticoccidial drug and tylosin was effective in controlling clinical disease. The addition of bacitracin (50 g/ton feed) is recommended as a preventative measure against the disease in quails. Adoption of hygienic measures such as wearing disposable shoes and gloves is very crucial to prevent spread of infection (Cooper et al., 2013).

Pasteurellosis

Pasteurellosis or Fowl Cholera (FC) in quails was first reported by Hinshaw and Emlen (1943) in captive California Valley quail (*Lophortyx caflfornicus*). Later on, the disease was described in different species of quails with a high mortality rate (13%) (Myint and Carter, 1988). *Pasteurella multocida* (*P. multocida*) serotype A:3 causing acute FC with high mortality was first reported in commercially raised Bobwhite quail in America (Panigrahy and Glass, 1982). Natural outbreaks of FC were reported in quails in Burma (Naveen and Arun, 1992), USA (Glisson et al., 1989), India (Chadran et al., 1995), Japan (Gowthaman et al., 2013), and Iraq (Hamad et al., 2012). In addition, signs and lesions of FC in quails were reported previously (Glisson et al., 1989; Bermudez et al., 1997; Goto et al., 2001; Odugbo et al., 2004; Akpavi et al., 2011). The mortality rate in natural outbreaks of FC

in quails can vary from 60% (Naveen and Arun, 1992; Miguel et al., 1998) to 99% (Bermudez et al., 1997). Japanese quails were susceptible to experimental infection with *P. multocida* serotypes A: 1, 3 and 4 and showed signs of weakness, inappetence, and sudden death. On pathological examination, petechial and ecchymotic hemorrhages on the heart and breast muscles as well as congestion of heart, liver, and lung were observed (Yakubu et al., 2015). Similar signs with 90% mortality were recorded in Japanese quails within 24 h post-inoculation with *P. multocida* serotype A: 4 (Akpavi et al., 2011). In India, the majority of *P. multocida* belonging to serotypes A: 1, 3 and 4 were associated with FC in quails (Kumar et al., 2004), however, *P. multocida* was isolated and molecularly identified from 330 apparently healthy quail chicks (8-day-old) with severe liver congestion and necrosis as well as bronchopneumonia (Babu Prasath et al., 2018). In Africa, a recurrent outbreak of FC in a Japanese quail farm was attributed to rats cohabiting quail houses (Mwankon et al., 2009). Treatment of Japanese quails infected with *P. multocida* using some antimicrobials (sulfonamides, oxytetracycline, doxycycline, neomycin, and norfloxacin) administered in the drinking water for five consecutive days was highly effective (Rigobelo et al., 2013).

Mycoplasmosis

Madden et al. (1967) reported the first isolate of *Mycoplasma gallisepticum* (*M. gallisepticum*) from a commercial Bobwhite quail flock with chronic respiratory disease. After, several quail cases of mycoplasmosis infection were reported (Tiong, 1978; Nascimento and Nascimento, 1986; Reece et al., 1986). Quails with mycoplasmosis indicate fibrinous perihepatitis, pericarditis and pleuritis, caseous materials in the air sacs and congested trachea (Barnes and Gross, 1997; Chauhan and Roy, 2008; Islam et al., 2016). Both *M. gallisepticum* and *M. synoviae* have been frequently isolated from quails as reported previously (Nascimento and Nascimento, 1986; Nascimento et al., 1997 and 1998). Infection with *M. gallisepticum* was serologically determined for the first time in 10-week-old quails with nasal discharge, mortality and swollen infraorbital sinuses in the Aydın region of Turkey (Türkyilmaz et al., 2007). In Bangladesh, the isolation rate of *Mycoplasma* from diseased and dead quails with chronic respiratory diseases was 5% (Islam et al., 2003, 2016). In a layer flock suffering from respiratory manifestation, mortality and egg production loss, *M. gallisepticum* were detected in 15 out of 17 (88.8%) quails and 12 out of 15 (80%) of birds showed mixed infections with *M. gallisepticum, P. multocida* and *E. coli* (Murakami et al., 2002). Concurrent infection of *M. gallisepticum* and *Subulura brumpti* was recorded in an 8-week-old Japanese quail breeder with mortality, caseous airsacculitis, and drop in egg production (Arulmozhi et al., 2018).

Infectious coryza

Infectious Coryza (IC), caused by *Avibacterium paragallinarum* (*A. paragallinarum*), is an upper respiratory disease in chickens and quails (Blackall and Hinz, 2008). Quail of all ages are susceptible to IC infection and the pathogen was isolated from naturally and experimentally infected Japanese quails (Cundy, 1965; Reece et al., 1981). Although quails are susceptible to IC, reports on isolation and identification of *A. paragallinarum* were rare (Blackall and Yamamoto, 1989). In a study, 53 Japanese quails representing from five commercial farms suffering typical IC, 8 isolates of *A. paragallinarum* were identified and molecularly characterized (Thenmozi and Malmarungan, 2013). In Indonesia, 5 out of 9 isolates (55.5%) of *A. paragallinarum* were identified from quails with typical sinusitis and facial edema. However, 3 out of 5 isolates were serologically identified as serovar B (Wahyuni et al., 2018). Recently, the migration pattern of *A. paragallinarum* was studied after experimental infection of Japanese quails and chicken. The results revealed prominent localization of the bacteria at 12 hours post-infection in nasal turbinates of quails and then decline in immunostaining intensity in the nasal tissue by 72 hours post-infection, indicating that the infection was resolved by the resident immune cells or by certain inherent innate immune factors in the nasal passage (Balouria et al., 2019).

Different antimicrobials have been used to treat IC infection, but many of them only lower the severity of the disease without complete curing the disease. Repeated treatments lead to the development of resistance to the used antibiotics (Tabbu, 2000). The antibiogram of *A. paragallinarum* in Japanese quails revealed complete (100%) resistance to ampicillin, neomycin, pefloxacin, co-cotrimoxazole, furazolidone, streptomycin, cephalexin and amikacin, 90% to gentamycin and 70% to oxytetracycline (Thenmozi and Malmarungan, 2013). Appropriate treatment requires antibiotic sensitivity tests to select effective and efficient drugs against the infection (Wahyuni et al., 2018). Diseased quails should be isolated from healthy ones and preventive sanitary measures, such as cleaning and disinfection of utensils, washing hands,

and change shoes during visiting the farm, should be applied (Blackall and Yamamoto, 1989; Blackall and Hinz, 2008)

Chlamydiosis

The correlation between the latent and lethal forms of avian chlamydiosis by using a Japanese quail as a model was examined. The results demonstrated that the latent chlamydial infection was converted to the lethal form in quails receiving cyclophosphamide treatment (Takashima et al., 1996). *Chlamydia psittaci* was histopathologically identified in a flock of Bobwhite quail aged 2-4 weeks old with 100% morbidity and 40-50% mortality, stunting and yellow/green diarrhea (Erbeck and Nunn, 1999). Tetracycline, erythromycin, azithromycin, and fluoroquinolones were proven to be effective against chlamydia infection (Takashima et al., 1996).

Mycotic diseases

Aspergillosis

Aspergillosis is a respiratory disease detected in a Japanese quail breeder with multiple grey lung nodules and airsacculitis (Basheer et al., 2017). *Aspergiluus flavus* (*A. flavus*) was associated with mycotic salpingitis in Japanese quails and white to grayish nodules (2-5 mm in diameter) were found on the serosal surface of oviduct (Singh et al., 1994). Also, natural and experimental aspergillosis caused by *A. fumigatus* and *A. flavus* was recorded in broiler quails (Gumussoy et al., 2004; Borah et al., 2010). In Bangladesh, the prevalence rate of aspergillosis was 3.99% among 476 diseased and dead quails. (Islam et al., 2016). Early treatment could be effective in case of mild or moderate lesions. Some medicaments including ketoconazole and amphotericin-B could be used to control aspergillosis (Dhama et al., 2012). Using copper sulfate for treatment of birds or litter can help in reducing fungal growth (Dyar et al., 1984). Birds severely affected should be culled from the flock. Strict sanitary and hygienic measures in the hatchery are very important (Beernaert et al., 2010). Good ventilation, good litter quality, proper stocking density and keeping feeders dry in the flocks are crucial to prevent *Aspergillus* growth (Kunkl, 2003).

Candidiasis

Experimental oral infection with *Candida albicans* (*C. albicans*) was successful in Japanese quails with severe macroscopic and microscopic hyperkeratosis along the digestive tract (Asrani et al., 1993). Cutaneous candidiasis with isolation of *C. albicans* was detected in the footpad lesions of Japanese quails (Sah et al., 1982). Adequate cleaning and disinfection, proper management, vitamin A supplementation and stopping of antibiotic administration are important for reducing the incidence of candidiasis (Dhama et al., 2013). Treatment with antifungal drugs such as nystatin, fluconazole or itraconazole is useful (Tiwari et al., 2011).

Mycotoxicosis

Mycotoxins are secondary toxic metabolites produced by fungal species under high temperature and high humidity during storage of poultry diets. Fumonisins B1 produced by *Fusarium* spp. is considered one of the most important mycotoxins that adversely affect the kidney tubules in the Japanese quail chicks fed with 200 ppm in diet for 21 days (Khan et al., 2013). Ochratoxin A is a fungal metabolite produced by *Penicillium* and several species of *Aspergillus* has embryotoxic, teratogenic and nephrotoxic effects on Japanese quail chicks at a dose of 16.5 mg/kg of body weight (Prior et al., 1976; Khan et al., 2013; Patial et al., 2013 a and b). Moniliformin, a water-soluble fungal metabolite produced by *Fusarium* spp., is associated with severe hypertrophic cardiomyopathy in Japanese quails (Sharma et al., 2012). Aflatoxin is an important toxin produced by *Aspergillus* especially *A. fumigatus, A. flavus, and A. parasiticus*. Quails are more sensitive to aflatoxins than other poultry species (Lozano and Diaz, 2006). Chang and Hamilton (1982) found decrease in body weight of laying Japanese quails fed diet containing aflatoxin concentrations (500 to 10,000 µg/kg) for 28 to 100 days. Japanese quails fed diets containing 25-100 µg/kg aflatoxin B1 showed poor feed intake, low egg weight and poor eggshell (Sawhney et al., 1973, Oliveira et al., 2002, Ogido et al., 2004, Oguz and Parlat, 2004, Sehu et al., 2005). The synergistic effect of aflatoxicosis and coccidiosis was studied in Japanese quails and the results revealed significant reduction in body weight and increase in oocyst production (Rao et al., 1990). Manafi (2018) mentioned that aflatoxin B1 (1.5mg/kg) had adverse effects on performance and biochemical parameters, gut physiology and immunity of laying Japanese quails and those alterations could be bypassed through using of herbal mycotoxin binder containing antioxidants, enzymes, and diatomaceous earth minerals. In addition, Sakamoto et al. (2018) reported that aflatoxin B1 (1500 µg/kg of diet) impaired hepatic function, productive performance and reduced egg weight in laying quails; however, addition of silymarin (500 g/ton) or adsorbent (1 kg/ton) was not able to ameliorate the adverse effects of aflatoxins on performance and

metabolism. It was observed that adverse effects of contamination of the diet with aflatoxin B1 in 21-day-old Japanese quails could be overcome by addition of probiotics containing *Bacillus*, which improved meat quality and microbial ecosystem of growing quail chicks (Kasmani et al., 2012 and 2018). *Nigella sativa* (black cumin seed) was found to be potent detoxifier for dietary aflatoxins in growing quails as inclusion of these seeds in the diet of quails induced significant improvement in immune responses, meat quality and intestinal *E. coli* populations (Rasouli-Hiq et al., 2016). Addition of glucomannan (2g/kg of the diet) overcome the adverse effects of aflatoxicosis in 60-day-old Japanese quails and reduced the pathological lesions in liver, kidneys, spleen, thymus glands and bursa of Fabricius (Yavuz et al., 2017). The role of dietary *Saccharomyces cerevisiae* inclusion to aflatoxin-contaminated diet was studied and the results indicated significant improvements in feed consumption, body weight and feed conversion ratio of Japanese quails (Parlat et al., 2001; Atalay, 2010). Citil et al. (2007) evaluated the protective capacity of L-carnitine to prevent the adverse effects of chronic aflatoxicosis in 8-week-old Japanese quails. Feeding of 2-week-old Japanese quail chicks on different doses of hydrated sodium calcium aluminosilicate partially protected the birds from the toxic effect of aflatoxicosis regarding measuring of some biochemical parameters, body performance and pathological lesions in different organs (Eraslan et al., 2004). Moreover, Migliorin et al. (2017) found that the use of adsorbent containing aluminosilicates, yeast cell wall, silymarin and bentonite after feeding of quails with aflatoxin-contaminated diet, prevented lipid peroxidation and free radical production and resulted in reduced histopathological lesions in liver.

Parasitic diseases

Coccidiosis

Coccidiosis is often a hidden disease in quails and causes severe economic losses due to increased mortality, decreased productivity and a predisposing factor for necrotic enteritis as a secondary bacterial infection (Simiyoon et al., 2018). Earlier in Oklahoma, *Eimeria* spp. was detected in 28% of Bobwhite quails (Alan Kocan et al., 1979). Three *Eimeria* spp. (*E. uzura*, *E. bateri* and *E. tsunodai*) have been identified in Japanese quails (Gesek et al., 2014). Natural infections with coccidiosis in Japanese quails exhibit signs of depression, anemia and blood mixed droppings (Teixeira et al., 2004; Simiyoon et al., 2018). On pathological examination, the caecum shows ballooning appearance with severe serosal and mucosal congestion and its lumen contained foul smelled necrotic materials admixed with blood (Umar et al., 2014; Anbarasi et al., 2016; Simiyoon et al., 2018). The histopathological changes revealed damage of intestinal villi and crypt epithelial cells with multiplying endogenous stages of *Eimeria* and a high number of oocysts (Teixeira and Lopes, 2002; Simiyoon et al., 2018). Arafat and Abbas (2018) detected that 34 out of 107 (31.78%) examined Japanese quail farms were positive for *Eimeria bateri*. Proper control measures should be considered in quail farms by avoiding water spillage, good stocking density, regular and hygienic disposal of litter and improving hygienic practices (Umar et al., 2014). Moreover, application of coccidiostats in the feed or coccidiocidal drugs in water is another way to control coccidiosis. Sokół et al. (2014) demonstrated that administration of toltrazuril in the drinking water completely eliminated *E. bateri* and induced significant reduction in *E. tsunodai* oocysts number in Japanese quails. A study conducted in Egypt compared the efficacy of coccidiocidal amprolium ethopabate and toltrazuril in the drinking water and prophylactic salinomycin and diclazuril as feed additives against *E. tsunodai* in Japanese quails, the results indicated that effects of curative drinking water treatments had the preference in comparison to prophylactic treatment (El-Morsy et al., 2016). The effect of feeding some herbal plants like *Matricaria chamomilla* on *E. bateri* infestation in 15-day-old quails was studied and the results revealed effective reduction of fecal oocyst shedding after treatment (Ahmadov et al., 2014). In addition, Arafat and Abbas (2018) concluded that oral immunization of 2-day-old Japanese quails with either 100 or 1000 sporulated oocysts of *E. bateri* improved weight gain and feed conversion rate as well as reduced diarrhea, intestinal lesions, and oocyst production. The FDA approved using of monensin sodium and amprolium as coccidiostats in the quail ration (El-Morsy et al., 2016). In conclusion, vaccination is a viable method to control coccidiosis in quails (Arafat and Abbas, 2018).

Other parasites

The examined intestine of Bobwhite quails revealed presence of a wide variety of nematodes, cestodes and protozoa including 27% *Subulara brumpti*, 4% *Heterakis gallinarum*, 6% unidentified cestodes, 45% *Trichomonas* spp., 30% *Chilomastix* spp., 27% *Eimeria* spp., 25% *Trichomonas gallinarum* and 7% *Histomonas melegridis* (Alan Kocan et al., 1979). The findings of a survey on 40 Bobwhite and Japanese quails conducted in Iran

(Shemshadi et al., 2014) indicated that 5% of the quails harbored *Raillietina echinobothrida* and *Raillietina cysticillus*, 20% quails harbored intestinal cryptosporidiosis and 32.5% quails had tracheal cryptosporidiosis. Microscopic examination on four young Bobwhite quails with anorexia, diarrhea, emaciation, and mortality as well as severe ulcerative enteritis, hepatic necrosis and peritonitis showed the presence of *Capillaria* spp., *Eimeria* spp. and *Histomonas* spp. (Roy et al., 2006). Cryptosporidiosis has been associated with high mortality in young quail with diarrhea (Hoerr et al., 1984 and 1986; Lindsay et al., 1991). Cryptosporidium infection also induced respiratory affections in quails (Tham et al., 1982). Mixed infections of *Cryptosporidium* spp., adenovirus (Tsai et al., 1998), *M. gallisepticum* (Murakami et al., 2002) and reovirus (Ritter et al., 1986) were previously recorded in quails. Experimental challenge of young Bobwhite quail with *Cryptosporidium* and reovirus showed an increase in the oocyst shedding; indicating the synergistic action of parasites and viral infections (Guy et al., 1987). Monte et al. (2018) demonstrated presence of different mixed protozoon parasites *Eutrichomastix globosus*, *Sphaerita* spp. and *Blastocystis hominis* in 12-week-old Japanese quails in Amazon region. An outbreak of histomoniasis caused by protozoan parasite *Histomonas meleagridis* was discovered in Bobwhite quails with high mortalities as well as typical cecal and liver lesions (McDougald et al., 2012). Application of sanitary measures including cleaning and disinfection of drinkers and feeders, all-in/all-out policy, control of rodents and insects, avoid mixing between different ages and species, and hygienic disposal of old litter are the essential (Alan Kocan et al., 1979). Specific treatment using anthelmintic or anti protozoon drugs is very important for disease eradication.

CONCLUSION

It is very important to give attention to quail production, as it could be considered an alternative to chicken meat or egg. Good management, prevention, and control of serious diseases affecting quails are very critical to improve production and immunity.

DECLARATIONS

Author's contribution

Wafaa Abd El-Ghany collected all the data, wrote and revised the manuscript.

Competing interests

The author has no conflict of interest.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 175-179, December 25, 2019

Research Paper, PII: S2322455X1900022-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.21

Systematic Program for Destroying of Flies' Population in Poultry Farm under Battery Cage Management in Russia

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Received: 11 Oct. 2019
Accepted: 19 Nov. 2019

ABSTRACT

Favorable conditions for development, reproduction, and accumulation of large amounts of zoophilous flies in commercial poultry farms are caused by incomplete compliance with veterinary and sanitary rules for growing in cage facilities. The purpose of the study was to test a systematic insecticidal program for destroying flies' populations using adulticide and larvicide drugs in poultry farms under battery cage management. The number of imago flies in hen houses was dynamically evaluated using flypapers, six flypapers in each hen house, situated in different levels above the floor. Flypapers were removed and the number of stuck insects was counted. The number of larvae was evaluated in dynamics by specimen testing from the floor area 10x10 cm, with weight of 3-5 g. The Quick Bayt WG 10% was applied to destroy the imago of flies. Baycidal® WP 25% was used against larvae of flies. Complex insecticide program Quick Bayt WG 10% + Baycidal® WP 25% provided the opportunity to destroy flies, with a significant difference in intensefficacy, (98.3 % for adult flies and 99.8 % for larvae). Furthermore, this program had a positive impact on economic indicators of meat production of broilers. The present study demonstrated high preventive efficacy and economical efficacy of complex program against flies under battery cage broiler management.

Key words: Adulticide, Economical Efficacy, Fly Larvae, Intensefficacy, Larvicide, Zoophilous Flies

INTRODUCTION

Poultry growing in premises which is free of endoparasites and ectoparasites is an important factor in improving national poultry breeding. Favorable conditions for development, reproduction, and accumulation of large amounts of zoophilous flies are due to the incomplete observance of veterinary and sanitary standards in commercial poultry housed in cage facilities. Several studies have established that flies are the carriers of infectious and parasitic diseases in humans, animals, and birds (Pokrovskiy and Zima, 1939; Veselkin, 1981; Kerbabaev, 2000).

The flies cause economic losses due to drop in egg production, reduction in chicken body weight gain, the premature slaughter of ill birds as well as damage to business reputation if fly larvae are founded in poultry and ready-to-cook foods (Safiullin et al., 2014).

For the proper management and successful protection against flies, it should be always remembered that the adult flies only include 15% of the population of flies, while larvae at different stages of development account for 85% of the population. For this reason, control of flies' population in poultry breeding requests simultaneous drug administration against adult and larval stages (Safiullin, 1995; Rules, 2002; Safiullin et al., 2011; Safiullin et al. 2017a; Safiullin, 2019). Hence, the aim of the present study was to evaluate the efficiency of complex insecticide (adulticide and larvicide) program to systematically eliminate flies' populations in broiler farms with the battery cage system.

MATERIALS AND METHODS

Ethical approval

The study was conducted in compliance with European Convention for the protection at vertebrate animals used for experimental and other scientific purposes (ETS 123, 1986) and the Russian Federation guidelines for good clinical practice (2003). Also, the current study was in accordance with the guidance for the

experimental evaluation of new pharmacological substances (Habriev, 2005).

Safety requirements

Safety requirements, such as work clothing, gloves, and rubber shoes, were considered when using insecticides. The drugs were applied in the houses during the preparation for birds' settlement.

Experimental design

The trial test was conducted in a poultry farm in the Vladimir Region, Russia, from July to September 2018. Laboratory studies were conducted in All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin, Moscow, Russia.

Studies were carried out in two similar hen houses, experimental and control, with a capacity of 84,445 birds. All hen houses were similar, equipped with three-storied battery cages installing in six levels and excrement channels along all hen house. Broiler chicken cross "Cobb-500" was in the hen houses when placement: 41,780 and 42,665 birds in experimental and control henhouse, respectively (Table 1). The average weight of broilers was 38 g in both henhouses when placement. Insecticide dressings of hen-houses and sanitary preparation of buildings were conducted according to the established procedure before the new batch of birds were stocked.

The primary insecticide program against flies in farms was applied using an adulticide drug on the basis of fipronil with a recommended dose. Adulticide agent of Quick Bayt® WG 10% (Bayer, Germany) was administered against adult flies in experimental henhouses. The drug dosage was selected according to the floor area. Two and one-half grams of Quick Bayt® WG 10% diluted in 20 ml of water per 1 m² of the floor. Treatment solution was applied to vertical surfaces by spraying using sprayer Gloriya (Gloria GmbH) with effective pressure, not more than 1 bar. Totally, about 30 % of walls area and other vertical surfaces where flies prefer to be (warm sunny walls, ventilation openings, window frames, and ceiling lights) were dressed by a vertical zigzag method up to a height of 2.5 m.

Floor dressing by larvicide drug of Baycidal® WP 25 % (Bayer, Germany), with a dose of 2 g per 1 m^2 floor surface, was performed immediately following wall dressing by the drug Quick Bay WG 10. Treatment solution was applied with the help of large-drop spray using DUK (Komarov's disinfecting units, GAM, Russia) at a rate of 200-300 ml per 1 m^2 of the floor and at proper places for flies' reproduction such as excrement channels, excrement hollows, carriers, and other places where rests of food, excrements may accumulate.

The number of imago flies in hen-houses was dynamically evaluated using flypapers (flycatcher "Mukholov-Kapkan", Avantari, Russia), six flypapers in each hen-house situated in different levels above the floor. After 24 hours, flypapers were removed and the number of stuck insects was counted. For the dynamic evaluating amount of larvae, the specimens obtained from the floor, with a surface area of 10×10 cm and weight of 3-5 g. In addition, contents of dung channels under battery cages were sampled using a special sampling device. Six aliquots were taken from each hen-house (Figures 1 and 2). At first, feces sampling was performed at the end of growing period of previous bird batch, in 24 hours after complex insecticide program, and then weekly for the entire duration of broiler growing.

Figure 1. Larvae and pupae of flies from the passage between the batteries

Figure 2. Larvae and pupae of flies from the litter channel

In total, flies were counted seven times during the study. The species of flies was established using the "Key to insects of the European part of the USSR" (Shtakelberg, 1969), "Key to insects of the European part of the USSR" (Bei-Bienko, 1970), the manual "Synanthropic two-winged fauna USSR" (Shtakelberg, 1956) and by using Zeiss "Primo Star" microscope.

The percentage of chickens' survival was determined by the ratio of the final number of birds to the initial number. The productivity and economic efficiency were evaluated by the mortality rate, average daily weight gain, and broiler feed costs.

Statistical analyses

Data on the number of imago and larvae flies were subjected to statistical analysis according to the method described by Plokhinsky (1978). Statistical analysis was performed using SAS/Stat software, version 9 (SAS Institute Inc., USA). Differences were considered to be significant for p-value <0.05.

RESULTS AND DISCUSSION

Two weeks before the end of the previous broiler party, the average number of imago flies and larvae was 1040 specimens in each flypaper and 145 specimens in each aliquot, respectively in henhouses where recommended dose of fipronil drug was administrated. This indicated a high risk for the farm.

This study indicated the following species: *Musca domestica domestica* (dominance index 93.38 %), *Protophormia terrae-novae* (dominance index 4.19 %) and *Licilla sericata* (dominance index 2.43 %) in the hen houses.

The average amount of adult flies decreased sharply in experimental hen-house (18 specimens per one flypaper) after dressing by the drug "Quick Bayt WG 10%" compared to the first measurement. "Quick Bayt WG 10%" in which imidacloprid (neonicotinoids group) is an active ingredient, indicated insecticidal activity against adult flies. At the same time, the number of imago flies was 1015 specimens after routine disinfection by fipronil in the control henhouse.

All aliquots taken from the floor and excrement channels in experimental henhouse were free of flies' larvae after dressing by the drug Baycidal WP 25%. Baycidal WP 25% has a larvicidal effect, interrupts the process of larval development, result in death before transformation into the adult stage. The active ingredient of triflumuron blocks chitin generation, which may be necessary for growing processes during larval molting in passing from one stage to another. As well as triflumuron has the ovicidal effect that leads to death of embryos and larvae hatched from the ootids (Howard and Wall, 1995). Moreover, it has been noted that triflumuron has a sterilization effect on adult flies (Broce and Gonzaga, 1987). Adult pubescent females failed to generate viable offspring after applying "Baycidal WP 25%". Also, this drug showed high efficiency against larvae of flies in pigsties (Safiullin et al., 2016).

Weekly studies showed a slightly residual number of adult flies and a complete absence of larvae during five weeks in the experimental henhouse. In the experimental henhouse, the number of flies and larvae remained very low close to zero during the whole broiler rearing period (38 days). Sustained duration of action of adulticide "Quick Bayt WG 10%" and larvicide "Baycidal WP 25%" did not give the opportunity to larvae to develop during the whole rearing period thus a significant reduction ($p<0.05$) in flies population was observed (Figures 3 and 4). Complex insecticide program "Quick Bayt WG 10%" + "Baycidal® WP 25%" provided the opportunity to destroy flies, with a significant difference in intensefficacy (98.3 % for adult flies and 99.8 % for larvae).

In control henhouse where larvicide was not applied and adulticide of fipronil was only used, larvae were recorded in a large number in excrement channels under cage batteries and on the floor where poultry feed and excrements were available. Consequently, flies' population was very high at these places and reduced slightly at the end of the period with the lowering of air temperature. Significant reduction in flies and larvae number in experimental henhouse during the whole cycle of growth showed a positive influence on chickens survival, weight gain, and feed-gain, which are consistent with data from other studies (Tashbulatov et al., 2016; Al Thabiani, 2017). Broiler chickens, which were grown in experimental henhouse, where larvicide and insecticide program had been conducted, exhibited better conditions for growth by reduction of stress caused by troublesome flies under the equal conditions with control henhouse in terms of environment, feeding, and drinking. In addition, it had a positive impact on productive economic parameters.

Chickens' survival throughout the growing cycle in experimental hen-house was 95.5%, and in control hen-house was 93.6%. The average daily weight gain of chickens in experimental and control hen-house was 58.6 g and 51.4 g, respectively ($P<0.05$) (Table 1).

Therefore, broiler chickens from the experimental hen-house where the complex insecticide program was

conducted, had 14% higher productivity compared to control where fipronil was only used as an insecticide.

The average slaughter weight was 2226.8 g and 1953.2 g in experimental and control hen-houses, respectively. The effective killing percentage was 73.7 % in both hen-houses. In monetary terms, a carcass from experimental group cost 268.2 Russian Ruble (RUB) and from control group cost RUB 253.3. The feed conversion ratio in the experimental and control group was 1.91 kg and 1.99 kg, respectively.

The cost of destroying flies and larvae in control where fipronil was administered was 8640 RUB, which was lower compared to the experimental hen house 12364 RUB. However, production and destroying efficacy was lower in the control house.

The economic effect of using complex insecticide program against adult flies and larval compared to fipronil drug was determined. The economic effect of administration of complex insecticide program against adult and larval flies was 14.9 RUB /one broiler, and 582.05 thousand RUB on all broilers in the experimental hen house.

Systematic program for destroying flies' population using adulticide and larvicide drugs in poultry farms under battery cage management provided high efficacy against adult flies and larvae. The average number of adult flies per 1 flycatcher was 18 specimens in the experimental henhouse for a five-week period after destroying, while in the control hen house was 1044 specimens. A similar result was for the larvae of flies. The number of larvae of flies after processing with "Baycidal® WP 25%" at different periods of research was as follows: during two weeks after processing, zero larvae; during the third and fourth weeks, one larva; and in fifth week zero larva. The average number of larvae was 0.3 specimens in the experimental henhouse. However, in the control henhouse there were 102 specimens. The present results are consistent with data of other researchers, which tested the combined use of Sulfak and Baicidal against adult flies and larvae in the pigsty (Safiullin and Ageev, 2016). Data are also consistent with the work of authors who evaluated Solfak with Baicidal in broiler farms with cage system (Safiullin et al. 2017b).

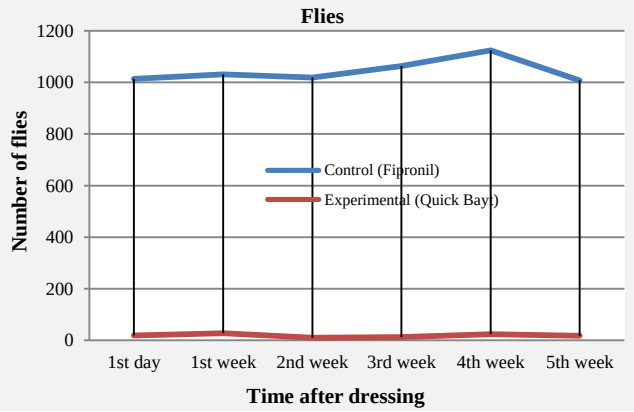

Figure 3. The number of adult flies during the five-week study period in the experimental and control henhouses, Russia

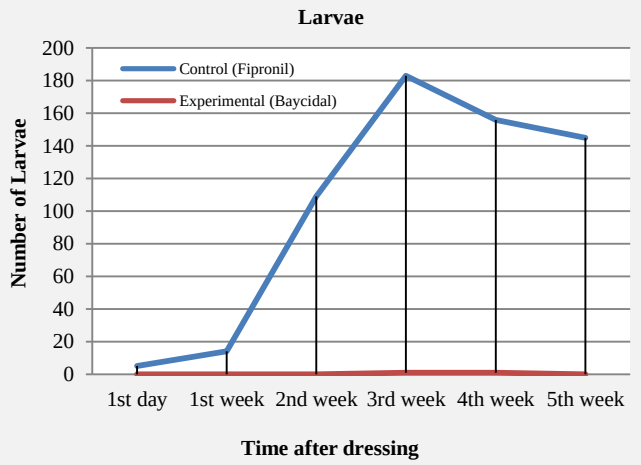

Figure 4. The number of larvae during the five-week study period in the experimental and control henhouses, Russia

Table 1. Effects of insecticides application against flies on economic and production parameters in broiler farms under battery cage management, Russia

Henhouses (groups)	Broilers' number when placement	The number of dead Chicken	Chicken's survival (%)	Average daily weight gain (g)	Average slaughter weight (g)	Killing percentage (%)	Cost per kilogram of carcass (RUB)	Feed for an increase of one kg of body weight (kg)	Expenditures connected with disinfestation of 1 hen-house (RUB)
Experimental*	41780	2716	95.5	58.6±1.67	2226.8	73.7	268.2	1.91	12364
Control**	42665	3584	93.6	51.4±1.84	1953.2	73.7	253.3	1.99	8640
Statistical significance					p<0.05				

* Insecticide program: Quick Bayt WG (water-soluble granules) 10% + "Baycidal WP (water-soluble powder) 25%; ** Adulticide drug on the basis of fipronil

CONCLUSION

In conclusion, the systematic program for destroying flies' population using an adulticide and larvicide drugs in poultry farm under battery cage management provided almost complete extermination of adult flies and larvae in difficult production conditions.

DECLARATIONS

Acknowledgments

We thank veterinarian experts of the poultry farm of the Vladimir Region which took an active part in conduction of these studies. Also we thank Bayer's employees for providing drugs for research. This study was supported by the All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin.

Authors' contributions

Rinat Tuktarovich Safiullin designed the performed the experiments. Radmir Rinatovich Safiullin and Ekaterina Olegovna Kachanova analyzed the results, drafted and revised the manuscript. Finally, all authors read and approved the final manuscript and consent to publish in JWPR.

Competing interests

The authors have declared that they have no competing interests.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 180-186, December 25, 2019

Research Paper, PII: S2322455X1900023-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.22

Effects of *Moringa oleifera* and *Garcinia kola* with or without Grits on Haematological and Serum Biochemical Parameters of Broiler Chickens

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Received: 14 Oct. 2019
Accepted: 20 Nov. 2019

ABSTRACT

The use of antibiotics as growth promoters in food animals has been banned due to the residual effects on final consumers which could lead to human health issues. The aim of the present study was to investigate the effects of two herbal feed additives with or without grits on hematological and serum biochemical parameters of broiler chickens. One hundred and forty-four, one-day-old, Cobb 500 broiler chicks were randomly assigned into six treatments (24 birds per treatment) with three replicates (eight bird per replicate). Six dietary treatments were formulated with the inclusion of *Moringa oleifera* Leaf Meal (MOLM), *Garcinia kola* Seed Meal (GKSM) and grits. The experimental rations contained diet without MOLM, GKSM and grits which served as treatment 1 (control), diet with MOLM at 1000ppm (treatment 2), diet with GKSM at 1000ppm (treatment 3), diet with grits at 1000ppm (treatment 4), diet with MOLM at 1000ppm + grits at 1000ppm (treatment 5) and diet with GKSM at 1000ppm + grits at 1000ppm (treatment 6). Blood samples were collected on 28 and 56 days of age for hematological and biochemical analysis. Data were subjected to analysis of variance in a completely randomized design. At the starter phase, red blood cells (1.15×10^{12} L) and white blood cells were significantly lowest in birds of first treatment. The birds that received treatment 6, had the highest glucose (131.50 g/dl) and high-density lipoprotein level (58.50 mg/dl). At the finisher phase, the lowest white blood cell count (10.95×10^{9}/L) and lymphocytes (60%) were recorded in treatment 6. Birds in treatment 3 indicated the lowest urea (2.05 mg/dl) and triglyceride (94.50 mg/dl). It can be concluded that diet supplemented with GKSM at 1000 ppm, increased high-density lipoprotein, and reduced triglyceride and low-density lipoprotein levels in serum of broiler chickens.

Key words: Blood parameters, Feed additive, *Garcinia Kola*, Grit, *Moringa oleifera*

INTRODUCTION

Feed additives used in poultry feed improve nutritive value, boost growth performance and feed conversion efficiency and lead to greater liveability and lower mortality in poultry. In the past, growth-promoting antibiotics were administered as feed additives and were associated with residues in the meat and eggs consumed by human, thus the usage of these agents banned or limited in many countries (Diarra et al., 2011; Gadde et al., 2017). Therefore, there is a need to develop new feed additives for replacing antibiotics because growth promoters and performance enhancers are of great importance to the poultry industry (Suresh et al., 2018).

Nowadays, veterinarians have turned attention towards alternative sources from natural ingredients such as herbs or phytogenic plants (phytobiotics) to replace antibiotics. There are reports on the beneficial effects of herbs used as feed supplements or medication in chickens (Ogbe et al., 2009). Certain bioactive chemicals in phytobiotics and herbs are responsible for their therapeutic benefits (Guo et al., 2003; Ogbe et al., 2009). Phytogenic plants generally contain chemical compounds such as saponins, tannins, oxalates, phytates, trypsin inhibitors, and cyanogenic glycosides which are known as secondary metabolites (Soetan and Oyewole, 2009). Secondary metabolites have high amounts of essential nutrients, vitamins, minerals, fatty acids, and fibers (Gafar and Itodo, 2011), therefore apply in nutrition and as pharmacologically-active agents (Soetan and Oyewole, 2009). Previous studies on herbal formulations as feed additives represent promising results in terms of weight

gain, feed efficiency, lowered mortality and increased liveability in poultry (Jahan et al., 2008).

It is reported that *Moringa oleifera*, known as the miracle tree, has many medicinal properties and antioxidant activity (Matthew et al., 2001; Ogbunugafor et al., 2011) and could be used as a substitute for conventional feedstuffs as it is a good source of vitamins and amino acids (Sarwart et al., 2002; Olugbemi et al., 2010). It is declared that *M. oleifera* promotes immune systems (Olugbemi et al., 2010). It has been reported that *M.oleifera* extract has antibacterial properties, thus has the potential to be investigated as a phytotherapeutic agent to combat the infectious organisms (Patel, 2011).

Garcinia kola or bitter kola, also known as African wonder nut, is used as food and herbal medicine (Adesanya et al., 2007). It contains phenolic compounds that possess anti-inflammatory, anti-microbial, anti-diabetic and antiviral properties (Adedeji et al., 2006). The presence of biflavonoids and xanthones that act as potent antioxidants, in *G. kola* seeds have been confirmed (Farombi et al., 2002; Oluyemi et al., 2007). Husain et al. (1982) reported antimicrobial activity of *G. kola* is due to kolanone whereas Iwu (1990) made the same observation with *G. kola* flavanone.

Grits are hard bits of stones, sand and small particles which birds used to enhance mechanical digestion by abrasion in the gizzard (Atteh, 2003). Grits can be classified into soluble fed and insoluble grits. Examples of soluble grits are limestone and oyster shells, which are easily dissolved in the gizzard, they also serve as a source of Calcium. The insoluble grits including silica, mica, and sand are non-digestible and are retained in the gizzard (Adeniji and Oyeleke, 2008). In addition, Atteh (2003) reported that grit improved feed utilization in the birds and average feed intake. To aid the gizzard, picking up a few stones as scavenging for feed is a natural behavior in chickens. These stones facilitate the mechanical digestion of materials that the chicken picks up (Salverson, 1996).

Therefore, the present study aimed to assess the effects of *G.kola* and *M.oleifera* as feed additives with or without sand grits on hematological and serum biochemical indices in broiler chickens.

MATERIALS AND METHODS

Study area

The present study was carried out in the poultry unit of Directorate of University Farms, Federal University of Agriculture Abeokuta, Nigeria. The area lies in the tropical rain forest vegetation zone. It is located 76 m above sea level with an average temperature of 34.7° C and a relative humidity of 82%.

Ethical approval

The present study was approved by the ethics and research committee of the Department of Animal Nutrition, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Sourcing and processing of test ingredients

The *M. oleifera* leaves were obtained from an established Moringa plot in Abeokuta, Ogun State, Nigeria. The *G kola* seeds were purchased from the Lafenwa market in Abeokuta, Ogun State and the sand grits of around 2 mm size were obtained from a beach in Ikorodu, Lagos State, Nigeria. Moringa leaves were washed with clean water and dried under shade and then powdered. The *M. oleifera* Leaf Meal (MOLM) stored in the dark in airtight plastic bags at ambient temperature.

The *G. kola* seeds were sun-dried and ground using hammer mill and referred to as *G. kola* Seed Meal (GKSM). The sand grits were sun-dried. All the test ingredients were stored in sacs until needed.

Experimental diets

Six experimental diets were formulated with the inclusion of herbal feed additives and grits as follows: basal diet (control; diet without herbal feed additives and sand grit; basal diet + MOLM (1000 ppm); basal diet + GKSM (1000 ppm); basal diet + grit (1000 ppm); basal diet + MOLM (1000 ppm) + grit (1000 ppm) and basal diet + GKSM (1000 ppm) + grit (1000 ppm). The starter and finisher diets were formulated as indicated in tables 1 and 2.

Study design

A total of 144 one-day-old, unsexed broiler chickens (Cobb 500) were purchased from a commercial hatchery (Zartech Hatchery, Ibadan, Nigeria). On arrival, all chickens were individually weighed and identified (using wing-tags). The birds were randomly divided into six treatment groups with three replicates (8 birds per replicate) in each group. Chickens were raised at 33 ±1 °C during the first and second weeks. The temperature was then reduced by 2 °C every week. Water and feed were provided *ad libitum*. All birds were reared on a deep litter in an open-sided house and kept under similar management conditions. The chicks were vaccinated against Newcastle disease and infectious bronchitis (LaSota strain and H120 strain at day 7 and 14 via

drinking water, respectively). Antibiotics were administered as therapeutic agents during the experiment. The study lasted for eight weeks.

Hematological and biochemical analysis

Blood samples were taken from the jugular vein of two chicks in each replicate on 28 and 56 days of age. The samples were transferred into Eppendorf tubes containing ethylenediaminetetraacetic acid (EDTA), as an anticoagulant to measure hematological parameters including Hemoglobin (Hb), Red Blood Cell (RBC), packed cell volume, White Blood Cells (WBC), and lymphocytes. In addition, for serum separation, blood samples were collected in non-EDTA tubes and allowed to clot for one hour at room temperature, and then centrifuged at 3,000 rpm for 20 min. Collected sera were stored in a deep freezer at –20°C until chemically analyzed. At the time of analysis, the samples were thawed and analyzed for total protein, albumin, glucose, total cholesterol, urea, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), triglyceride, Aspartate Transaminase (AST) and Alanine Transaminase (ALT).

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test when ANOVA analysis was significant. Data analysis was performed using SPSS 16.0 (SPSS Inc., USA). A p-value of 0.05 or less was considered significant.

Table 1. Composition of experimental diets for Cobb 500 broiler chickens aged 0-4 weeks

Ingredients (%)	Basal diet (Control)	MOLM	GKSM	Grits	MOLM + Grits	GKSM + Grits
Maize	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	8.00	8.00	8.00	8.00	8.00	8.00
Soybean meal	22.00	22.00	22.00	22.00	22.00	22.00
Groundnut cake	10.30	10.30	10.30	10.30	10.30	10.30
Palm Kernel Cake	2.00	2.00	2.00	2.00	2.00	2.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin & Mineral Premix*	0.25	0.25	0.25	0.25	0.25	0.25
MOLM	-	+	-	-	+	-
GKSM	-	-	+	-	-	+
Grits	-	-	-	+	+	+
Total	100	100	100	100	100	100
Calculated Chemical Composition						
Metabolizable energy (Kcal/Kg)	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13
Crude protein (%)	22.44	22.44	22.44	22.44	22.44	22.44
Crude fibre (%)	4.03	4.03	4.03	4.03	4.03	4.03
Fat (%)	4.29	4.29	4.29	4.29	4.29	4.29
Ca (%)	1.66	1.66	1.66	1.66	1.66	1.66
P (%)	0.82	0.82	0.82	0.82	0.82	0.82

*Premix to provide the following: Vitamin A 12,000,000I.U; Vitamin D3 3,000.000I.U; Vitamin E 30,000mg; Vitamin K 2,500mg; folic acid 1,000mg; Niacin 40, 000mg; Cal Pan 10,000mg; Vitamin B12 20mg; Vitamin B12,000mg; Vitamin B6 3,500mg; Biotin 80mg; Antioxidant 125,000mg; Cobalt 250mg; Selenium 250mg; Iodine1,200mg; Iron 40,000mg; Manganese 70,000mg; Copper 8,000mg; Zinc 60,000mg; Chlorine 200,000mg. +: 1000 ppm, MOLM: *Moringa oleifera* Leaf Meal, GKSM: *Garcinia kola* Seed Meal

Table 2. Composition of experimental diets for Cobb 500 broiler chickens aged 4-8 weeks

Ingredients (%)	Basal diet (Control)	MOLM	GKSM	Grits	MOLM + Grits	GKSM + Grits
Maize	54.00	54.00	54.00	54.00	54.00	54.00
Wheat offal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean Meal	16.00	16.00	16.00	16.00	16.00	16.00
Palm kernel cake	3.00	3.00	3.00	3.00	3.00	3.00
Groundnut cake	9.30	9.30	9.30	9.30	9.30	9.30
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin & Minreal Premix*	0.25	0.25	0.25	0.25	0.25	0.25
MOLM	-	+	-	-	+	-
GKSM	-	-	+	-	-	+
Grits	-	-	-	+	+	+
Total	100	100	100	100	100	100
Calculated Chemical Composition						
Metabolizable energy (Kcal/Kg)	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33
Crude Protein (%)	20.24	20.24	20.24	20.24	20.24	20.24
Crude Fiber (%)	3.98	3.98	3.98	3.98	3.98	3.98
Fat (%)	4.27	4.27	4.27	4.27	4.27	4.27
Ca (%)	1.65	1.65	1.65	1.65	1.65	1.65
P (%)	0.82	0.82	0.82	0.82	0.82	0.82

*****Premix to provide the following: Vitamin A 12,000,000I.U; Vitamin D3 3,000.000I.U; Vitamin E 30,000mg;Vitamin K 2,500mg; folic acid 1,000mg; Niacin 40, 000mg; Cal Pan 10,000mg; Vitamin B12 20mg; Vitamin B12,000mg; Vitamin B6 3,500mg; Biotin 80mg; Antioxidant 125,000mg; Cobalt 250mg; Selenium 250mg; Iodine1,200mg; Iron 40,000mg; Manganese 70,000mg; Copper 8,000mg; Zinc 60,000mg; Chlorine 200,000mg. +: 1000ppm, MOLM: *Moringa oleifera* Leaf Meal, GKSM: *Garcinia kola* Seed Meal

RESULTS

Effects of experimental diets on the hematological and serum biochemical indices during the starter phase (0-4 weeks)

Table 3 shows the main effects of herbal feed additives and grits on hematological and serum biochemical indices of the birds in 4th week. The Hb, RBC, and WBC were significantly influenced by herbal feed additives and grits ($p<0.05$). The birds fed on the control diet and GKSM + grits recorded significantly lower Hb values (7.15 and 7.75g/dl; respectively) ($p<0.05$) while other treatment groups had comparable values. The highest and lowest value for RBC were recorded in birds fed on GKSM and control diets; respectively ($p<0.05$). The birds fed basal diet + grits recorded significantly higher value (13.6 $\times 10^9$/L) for WBC ($p<0.05$), followed by groups fed GKSM while others had comparable lower values. Other parameters measured were not significantly influenced by herbal feed additives and grits. The glucose, urea, and HDL were significantly affected by herbal feed additives and grits. The birds fed grits indicated significantly the highest glucose value ($p<0.05$) while those fed MOLM had the lowest value. The birds fed on control diet recorded significantly the highest urea value (2.35 mg/dl; $p<0.05$) whereas other values were comparable across the treatments. The birds fed MOLM + grits and GKSM + grits recorded similar HDL levels (58.50 mg/dl), which were not significantly higher than other treatment groups.

Effects of experimental diets on the hematological and serum biochemical indices during the finisher phase (4-8 weeks)

Table 4 displays the effects of herbal feed additives and grits on hematological and serum parameters of broiler chickens in 8th week. There were significant differences in most of the hematological parameters measured. The values of WBC were significantly lower ($p<0.05$) in birds fed MOLM + grits and GKSM + grits

(10.65×10^9/L and 10.95×10^9/L; respectively) compared to control group which had the highest value of 14.05×10^9/L. Groups fed grit and GKSM +grits had heterophils values of 39.50 and 40.00%, respectively, and were significantly higher than other treatment groups ($p<0.05$). The lymphocytes in the blood of the birds fed on MOLM + grits and GKSM + grits were significantly lower compared to the control birds ($p<0.05$). Monocyte value (1.00%) was highest in the control group while the least value (0.0001) was recorded in other groups except group fed MOLM + grit with a monocyte value of 0.5%. The MCV value in MOLM treatment (182 fL) was significantly higher than the other groups. Other parameters measured were not significantly influenced by dietary treatments.

Feed additives and grits supplementation influenced some serum parameters including globulin, AST, urea, triglyceride, LDL, and VLDL. The lowest and highest globulin levels were achieved in the birds fed on control diets and GKSM; respectively ($p<0.05$). The AST concentration in birds fed on GKSM was significantly higher (62.50 U/L; $p<0.05$) than those fed MOLM + grits which had the lowest value of 56.00 U/L. The highest urea value was observed in birds fed MOLM + grits ($p<0.05$) while those fed control, MOLM, and GKSM had similar values. The lowest triglyceride value was achieved in birds fed GSKM ($p<0.05$) while birds in MOLM, grits, and GKSM + grits treatments had similar values. The LDL level in MOLM + grits treatment was significantly higher compared to GSKM + grits treatment ($p<0.05$). The lowest level of VLDL was achieved in birds fed MOLM ($p<0.05$) while those fed GKSM, grits and GSKM + grits had similar values.

DISCUSSION

Blood parameters are considered valuable indicators for health status (Rehman et al., 2017). The values of Hb, RBC, and WBC obtained in the present study were within the normal ranges reported by Morton et al. (1993). The numerical differences observed in the Hb and RBC levels in birds fed herbal feed additives solely or with grits suggests that the diets were better utilized and assimilated into the bloodstream for use by the birds. Hematological studies in birds demonstrated that RBC and other parameters such as Hb vary among bird species and are affected by diet contents, (Odunsi et al., 2002) physiological and environmental conditions (Alodan and Mashaly, 1999). Olugbemi et al. (2010) reported that hemoglobin was not significantly affected when broiler chickens were fed with *Moringa oleifera*.

The glucose concentrations in this study were within the normal range reported by Mitruka and Rawsley (1977). Glucose is one of the metabolites which represent the energy status of the animal. Normal glucose levels in birds indicate adequate synthesis in the liver from propionate, a major glucose precursor (Houtert, 1993). The results are in line with the findings of Udenze et al. (2012b) who reported that *G. kola* powder reduces glucose concentrations in diabetic animals and normalize glycemia at the highest dose.

In the present study, the high HDL level at starter phase as well as lower values of triglycerides, LDL, and cholesterol at the finisher phase indicated that the diets containing *G. kola* have good lipid-lowering agents, which is associated with a reduced risk of cardiovascular diseases (Ouyang et al., 2016). The reduction of triglycerides and LDL could be attributed to the inhibitory effect of *G. kola* seed on the accumulation of lipid droplets in adipocytes (Noboru, 2001). Ali et al. (2007) found that adding thyme to hen's ration significantly decreased plasma HDL, total cholesterol, triglycerides, and total lipids. Contrarily, Bolukbasi et al. (2006) reported that dietary thyme oil increases plasma concentration of triglycerides, LDL-cholesterol and HDL-cholesterol in broiler chickens.

CONCLUSION

It is concluded that diet supplemented with GKSM at 1000ppm increases high-density lipoprotein, reduces triglyceride and low-density lipoprotein levels in serum of broiler chickens.

DECLARATIONS

Acknowledgments

This study was supported by the Animal Nutrition Laboratory of the Department of Animal Nutrition, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Competing interests

The authors have declared that no competing interest exists.

Authors' contributions

Adejola YA designed the analysis, collected the data and wrote the manuscript. Sobayo RA supervised and designed the analysis. Muhammed SB contributed analysis tool and performed the analysis. Ayoola AA collected the data and performed the analysis. Jinadu KB wrote the paper.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 187-195, December 25, 2019

Research Paper, PII: S2322455X1900024-9
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DOI: https://dx.doi.org/10.36380/jwpr.2019.23

The Evaluation of Dietary Addition of Palm and Coconut Oils in Steaming Tomato (*Lycopersicon esculentum*) Waste Powder on Digestibility of Crude Fiber and Retention of Lycopene and Nitrogen in Broiler Chickens

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Received: 18 Oct 2019
Accepted: 26 Nov 2019

ABSTRACT

Lycopene is a powerful antioxidant present in tomatoes and other vegetables and fruits. Present research was carried out to evaluate lycopene and nitrogen retention and crude fiber (CF) digestibility of steaming tomatoes waste powder which was combined with oil. Tomatoes waste in this experiment were local fresh tomato rejected from tomato field around West Sumatera province, Indonesia. The experimental factors were included the type of oil (palm and coconut oils) and dosage of oils (0.25, 0.5, 0.75, 1, and 1.25 %), and each treatment was replicated three times. The results indicated there was an interaction between the type of oil and dosage of oil on lycopene retention, and CF digestibility, while the type of oil and dosage of oil affected lycopene retention significantly. The dosage of oil also influenced lycopene retention, nitrogen retention, CF digestibility significantly. The addition of coconut oil in steaming tomato waste powder increased lycopene and nitrogen retention, and CF digestibility higher than the addition of palm oil to steaming tomato waste powder in broiler chickens. The lycopene and nitrogen retention, and CF digestibility of steaming tomato waste powder added 0.5% coconut oil was the best level for lycopene and nitrogen retention, and CF digestibility in broiler chickens.

Key words: Coconut oil, Crude fiber digestibility, Lycopene retention, Nitrogen retention, Palm oil, Tomatoes waste

INTRODUCTION

Recently, researches on poultry nutrition were conducted to find cheaper and high quality feeds. One of the famous active compound in tomato is lycopene. This compound is beneficial for human and livestock health (Bramley, 2000). Lycopene was known as a substance that has high antioxidant ability (Dewanto et al., 2002; Toor and Savage, 2005; Gupta et al., 2011; Surai, 2016). In addition, lycopene also has an important role to reduce cholesterol level in the body (Palozza et al., 2012). Lycopene can inhibit HMG-CoA reductase to synthesize mevalonate from HMG-CoA, resulting in reducing of cholesterol level in the body (Palozza et al., 2012).

Previous research showed lycopene and tomato wastes have been widely used as feed mixtures in poultry. This research shown, that semen production and viability were affected by lycopene supplementation in broiler breeder (Mangiagalli and Taylor, 2010). It could reduce cholesterol content in meat (Mahata et al. 2016 a), and in serum of broiler chickens (Mahata et al., 2016a) and laying hen (Mahata et al., 2016b), and it also improved poultry immunity status (Olson et al., 2005; Mangiagalli and Taylor, 2010; Sun et al., 2015). Generally, lycopene structure in fresh tomato is in trans form, and it is very stable so that it is difficult to absorb by animals and humans digestive tract (Unlu et al., 2007; Knockaert et al., 2012; Meroni and Raikos 2018). Handayani et al. (2018) reported that to increase the availability of cis-lycopene and its absorption in poultry digestive tract, fresh tomato should be treated by steaming for 12 minutes at temperature 98 ^{0}C. This treating will change the structure of lycopene in fresh tomato from trans structure to cis structure.

To cite this paper: Handayani UF, Wizna, Suliansyah I, Rizal Y and Mahata ME (2019). The Evaluation of Dietary Addition of Palm and Coconut Oils in Steaming Tomato (*Lycopersicon esculentum*) Waste Powder on Digestibility of Crude Fiber and Retention of Lycopene and Nitrogen in Broiler Chickens. *J. World Poult. Res.*, 9 (4): 187-195.

One of the characteristics of lycopene is lipid soluble (Clinton, 1998; Shi and Maguer, 2000; Colle et al., 2010; Colle et al., 2013; Trujillo and Mc-Clements, 2016), so that to increase lycopene absorption in the digestive tract, it must be mixed with lipid. Clark et al. (2000) found that olive oil was better than corn oil in improving lycopene absorption in the rat, because the corn oil is rich with the Polyunsaturated Fatty Acid (PUFA) like linoleic acid, while olive oil is rich with the Monounsaturated Fatty Acid (MUFA) like oleic acid. Furthermore, Clark et al. (2000) explained that lycopene is a part of carotenoid with less absorption of carotenoid in digestive tract of rat due to carotenoid oxidation which promotes by high PUFA content in corn oil and transfer of carotenoid to micelles from lipid emulsions containing large amounts of PUFA and bile salt will reduce, so that the absorption of lycopene mixed with corn oil was less than absorption of lycopene mixed with olive oil. On the other hand, Colle et al. (2012) reported the *in vitro* experiment about the addition of 5% different lipid (coconut oil, palm oil, cocoa butter, olive oil, sunflower oil, and fish oil) to row tomato pulp were significantly affected the lycopene bioaccessibility. Furthermore, in the second experiment Colle et al. (2012) added of coconut oil, olive oil, and fish oil of 0, 1, 2, 5, and 10% to row tomato pulp, and the highest lycopene bioaccessibility for olive oil and fish oil was after adding 2% and 1% of oil respectively, while the highest lycopene bioaccessibility for coconut oil was as much as 10%.

Palm oil and coconut oil are rich with saturated fatty acids like palmitic acid and lauric acid respectively (Dinicolantonio and O'Keefe, 2017). Both oils (palm oil and coconut oil) have potential to dilution of lycopene for increasing it's bioaccessibility by *in vitro* study (Colle et al., 2012), but the research that specifically investigates lycopene absorption by *in vivo* study in poultry is limited. Moreover, the digestibility of crude fiber and nitrogen retention from steaming tomato waste powder are important to evaluate, because maybe the steaming and oil addition to tomato waste will affect the content and structure of protein and crude fiber in tomatoes. According to Mahata et al. (2012), physical treatment (steaming) degraded crude fiber bond in juice waste mixture, and it also decreased crude fiber content. The addition of lipid in diet of poultry could reduce the rate of feed digestion in digestive tract, and it improved digestibility of feed nutrient (Baiao and Lara, 2005; Latshaw, 2008; Rizal, 2013). Therefore, this study was performed to evaluate the effects of coconut and palm oil addition in steaming tomato waste powder on lycopene and nitrogen retention, and crude fiber digestibility.

MATERIALS AND METHODS

Ethical approval

The animal experiments were carried out in accordance with the guidelines laid by institutional Ethics committee for the care of animals and were approved by Animal Ethics Committee of the Universitas Andalas, Padang, Indonesia with No:574/KEP/FK/2019.

Coconut oil and palm oil

Palm oil from one of the local brand (merk Rose Brand from Rose Brand inc, Jakarta, Indonesia) with the purity as much as 99.81%. Coconut oil was prepared by extraction of coconut milk from a mature coconut, and then it boiled until the oil from coconut milk was produced. Furthermore, the oil was separated from coconut cake by filtration. The purity of coconut oil as much as 99.85%. The fatty acid content of each coconut oil and palm oil presented in table 1.

Steaming tomato powder waste

The type of tomato used in this experiment was mature tomato (*Lycopersicon esculentum*) waste. It was collected from rejected tomatoes at an agricultural field in Alahan Panjang, West Sumatera province, Indonesia. The tomato waste was steamed in the boiled water at 98°C for 12 min (Handayani et al., 2018). Furthermore, steaming tomatoes wastes were dried in the oven at temperature 60°C for three days (Handayani et al., 2018) and then ground to be a powder. The nutrient content of tomato powder shown in table 2.

Table 1. Fatty acid composition of coconut oil and palm oil

Fatty acid	Coconut oil	Palm oil
C4 (butyric acid) (%)	0	0
C6 (caproic acid) (%)	0	0
C8 (caprylic acid) (%)	0,23	0
C10 (capric acid) (%)	4,53	0
C12 (lauric acid) (%)	55,28	0,26
C14 (myristic acid) (%)	21,8	1,26
C16 (palmitic acid) (%)	7,34	35,6
C18 (Stearic acid) (%)	3,13	5,07
C18:1 (oleic acid) (%)	4,97	39,91
C18:2 (linoleic acid) (%)	2,12	16,1

Source: analysis at the Agro-Industrial Center Central Laboratory, at Bogor city, West Java, Indonesia, 2018

Table 2. Nutrient content of tomato powder

Nutrients	Amount
Crude protein (%)	10,88
Crude lipid (%)	3,85
Crude fiber (%)	11,92
Calcium (%)	0,26
Phosphorus (%)	0,69
Metabolism Energy (KCal/kg)	1596
Methionine (%)	0,25
Lysin (%)	0,83
Lycopene (mg/100g)	52,10

Experimental animals

Totally 60 broiler chickens (strain MB 202 from Japfa comfeed inc. Indonesia) at seven weeks of age were purchased from a local broiler farm. Broiler chickens divided randomly into 10 groups combination treatment from type of oil and dosage of oil (6 bird /group) with three replicates each (2 bird/ replicate). Another birds as much as 6 birds were prepared for control treatment (tomato steaming powder without oil) and 4 other bird were used for collecting excreta from endogenous nitrogen. Then birds were kept in wire cages, and each cage was provided with water troughs and tray to collect excreta. The control treatment and endogenous nitrogen treatment were not statistically analysed. Endogenous nitrogen treatment was used for calculate retention nitrogen.

Experimental design

The experiment was performed in a 2×5 factorial (Steel and Torrie, 1980) arrangement of lipid addition to steaming tomato waste powder in completely randomized design (totally 10 treatments), and each treatment was replicated three times. The first factor was different type of oil (coconut oil and palm oil), and the second factor was different dosage of oil (0.25, 0.5, 0.72, 1, and 1.25 % in feed). The combination of treatments as follows: Addition coconut oil at different dosages as much as 0.25, 0.5, 0.75, 1, and 1.25%. Also addition of palm oil at different dosages as much as 0.25, 0.5, 0.75, 1, and 1.25% was considered.

Forced feeding

Before forced feeding, all birds were fasted for 32 hours by modified method of Sibbald (1976) to make empty digestive tracts of the birds. The water was prepared *ad libitum*. 20g of steamed tomato waste powder prepared in paste (20 ml water mixed with the tomato powder until to be paste). Then tomato paste was added to oil as much as appropriate with each treatment (0.25, 0.5, 0.72, 1, and 1.25 % in feed), and then each bird was forced feeding. Force-feeding was accomplished by inserting a gastrointestinal tube into the esophagus. The tomato paste pushed into the crop with a syringe rod. After force-feeding, the birds were returned to their cages. A tray was placed under each cage to collect excreta. Excreta samples from each bird was collected at 48 hours after forced feeding by modified method of Sibbald (1976). Excreta on the tray was moved to another tray every three hour and contaminants of excreta, such as feathers were removed carefully before excreta were dried. The excreta were dried in the oven at temperature 60 ^{0}C for 48 hours, ground to be powder for lycopene retention, nitrogen retention, and crude fiber digestibility analysis.

Measurement of lycopene

Lycopene was analyzed by modification from Sharma and Le Maguer (1996) method. 1.25 g of excreta powder from each bird was placed in Erlenmeyer 250 ml, covered with aluminum foil, and added with 12.5 ml of mixed solution hexane: acetone: ethanol (2: 1: 1, v/v/v). That mixture solution was made with mixing 6.25 ml of hexane, 3.125 ml acetone, and 3.125 ml of ethanol. Excreta powder that has been added with a mixed solution was shaken for 30 minutes with a magnetic stirrer, then separated by funnel separate, and 10 ml of distilled water was added, and then shaked again for 15 minutes. At last, the the polar and non-polar layers were separated by separating funnel and all the top layer (non-polar) poured to a 25ml measuring flask, then added N-hexane until mark boundaries. The total lycopene content of non-polar layer with UV-Vis spectrophotometry (UV-1800 Shimadzu Kyoto, Japan) at a wavelength of 417 nm was determined. Lycopene level was calculated by standard regression.

Measurement of lycopene retention

Lycopene Retention (LR) was calculated by the modification method of Jain (1999) as follows:

$$LR\ (\%) = \frac{\sum \text{lycopene consumption} - \sum \text{lycopene in excreta}}{\sum \text{lycopene consumption}} \times 100\%$$

Measurement of nitrogen and crude fiber

Nitrogen and Crude Fiber (CF) of excreta from each bird was analyzed by proximate analysis (AOAC, 1990). Measurement of nitrogen retention and CF digestibility, nitrogen retention was calculated by the method of Sibbald (1985) and CF digestibility were calculated by the method of Mujahid et al. (2003) with few modifications as follow:

$$\text{Nitrogen Retention}(\%) = \frac{\Sigma \text{ N consumption} - (\Sigma \text{ N excreta} - \Sigma \text{ N Endogenous})}{\Sigma \text{ N consumption}} \times 100\%$$

$$\text{CF digestibility}\ (\%) = \frac{\text{CF consumption} - \text{CF excreta}}{\text{CF consumption}} \times 100\%$$

Analysis of data

Data were statistically analyzed by one-way analysis of variance. Differences among treatments were determined with Duncan's multiple range test (DMRT) according to Steel and Torrie (1980). The significant differences was indicated at P<0.05.

RESULTS

Lycopene retention from steaming tomato waste powder which was added with different types of oil (coconut and palm oils), and combined with different dosage of both of oils (0.25, 0.5, 0.75, 1, and 1.25%) was shown in table 3. There was interaction (P< 0.05) between type and dosage of oil on lycopene retention. The interaction between dosage of oil at 0.25% with the type of coconut oil and palm oil showed no significant (P> 0.05) effect on lycopene retention, as well as the interaction between dosage of oil at 0.5% with both type of oils did not significantly (P> 0.05) affect the lycopene retention. So there were no interactions (P> 0.05) between dosage of oil (0.25% and 0.5%) with the type of both different oils in lycopene retention. The interaction of both palm and coconut oil dosages at 0.75, 1, and 1.25% indicated significant (P<0.05) effects on lycopene retention.

The result of nitrogen retention of steaming tomato waste powder with different oil types, and different dosage of oil are summarized in table 4. It was obtained that there was no interaction (P> 0.05) between types of oil (palm and coconut oils) and dosage of oil (0.25, 0.5, 0.75, 1, and 1.25%) on nitrogen retention, but nitrogen retention was affected by the type of oil (palm and coconut oils) significantly (P<0.05), and also the dosages of oil (0.25, 0.5, 0.75, 1, and 1.25%) showed significant (P<0.05) effects on nitrogen retention. In this study, the dosage of oil at 0.5% was the best dosage to increase nitrogen retention compared to other oil dosages (0.25, 0.75, 1, and 1.25%).

There was significantly interactions (P< 0.05) between the types of oil with the dosage of oil on CF digestibility (Table 5). That shows the type of oil and how much dosage of oil added affect CF digestibility in the digestive tract of broiler. CF digestibility was affected by types of oil (palm and coconut oils) significantly (P<0.05), and also the dosage of oil showed significant (P<0.05) effects on CF digestibility. The dosage of both of oils at 0.25% in diet showed the CF digestibility (24.50%) lesser than the dietary dosage of both of oils at 0.5% (32.16%). The highest CF digestibility was found in treatment coconut oil at the dosages of 0.25% and 0.5%. Increasing dosages of oil (0.75, 1 and 1.25) caused reduction in CF digestibility in both types of oil. It means that the dosages of oil should be added not more than 0.5% in other to obtain the best CF digestibility.

Table 3. Effect type and dosage of oil (coconut and palm oil) in steaming tomato waste on lycopene retention of broiler chicken

Type of oil	Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	47.19^{bc}	57.49^{a}	47.16^{bc}	46.21^{bc}	41.85^{c}	47.98^{B}
Coconut oil	49.97^{b}	61.74^{a}	57.77^{a}	56.46^{a}	58.16^{a}	56.82^{A}
Means	48.58^{b}	59.62^{a}	52.46^{b}	51.34^{b}	50.00^{b}	

$^{a-c}$Means values in the same row bearing different superscripts are significantly different (P<0.05), $^{A-B}$Means values in the same column bearing different superscripts are significantly different (P<0.05), SEM=1,71

Table 4. Effect type and dosage of oil (coconut and palm oil) in steaming tomato waste on on nitrogen retention of broiler chicken

Type of oil	Dosage of Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	34.67	46.16	35.01	26.79	22.38	33.00^{B}
Coconut oil	54.43	62.39	47.69	46.79	38.93	50.05^{A}
Means	44.55^{b}	54.28^{a}	41.35^{b}	36.79^{c}	30.65^{d}	

$^{a-e}$Means values in the same row bearing different superscripts are significantly different (P<0.05), $^{A-B}$Means values in the same column bearing different superscripts are significantly different (P<0.05), SEM= 1,37

Table 5. Type and dosage of oil (coconut and palm oil) in steaming tomato waste on on crude fiber digestibility of broiler chickens

Type of oil	Dosage of Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	15.65^{c}	28.05^{b}	11.60^{cde}	11.35^{de}	9.29^{e}	15.19^{B}
Coconut oil	33.36^{a}	36.26^{a}	15.34^{cd}	14.82^{cd}	13.03^{cde}	22.56^{A}
Means	24.50^{b}	32.16^{a}	13.47^{c}	13.09^{c}	11.16^{c}	

$^{a-e}$Means values in the same row bearing different superscripts are significantly different (P<0.05), $^{A-B}$Means values in the same column bearing different superscripts are significantly different (P<0.05), SEM=1,24

DISCUSSION

Based on the result in this experiment, there was interaction between type and dosage of oil on lycopene retention. The lycopene retention at the dosage of 0.25% and 0.5% for both types of oils was not different. While, lycopene retention in tomato waste powder of steaming tomato with coconut oil at dosages of 0.75, 1, and 1.25% was as much as waste powder of steaming tomato with coconut oil at dosages of 0.5%. Lycopene retention in waste powder of steaming tomato with palm oil at dosages of 0.75, 1, and 1.25% decreased lycopene retention in comparing with 0.5% dosage. This condition showed when the dosage of oil is low (less than 0.5%), fatty acid from coconut oil and palm oil (Table 1) are able to solve the lycopene in micelle (an aggregate of molecules in a collodial solution) of poultry digestive tract. Therefore lycopene absorption can be easily in digestive tract. When the oil dosage is high (more than 0.5%) in waste powder of steaming tomato, the medium chain saturated fatty acid in coconut oil appear better than palm oil which contains long chain saturated fatty acid to solve lycopene in micelle. According to Li et al. (2011) when in digestive tract the lipid load is high, triglycerides with long chain fatty acids will hydrolysis by enzyme (lipase), although this hydrolysation was lesser for triglycerides with medium chain fatty acids. Acording to Agarwal and Rao (1998) Lycopene is a part of carotenoid. An experiment on simulation of gastric-duodenal fluid that showed the dilution of carotenoid in oil which not combined with micelles is higher than carotenoid in oil which combained with micelle (Malaki et al., 2010). That is the reason why the lycopene from steaming tomato waste powder which added with palm oil which contains long-chain fatty acid less absorption when high lipid load in digestive tract in this experiment. According to Huo et al. (2007) the addition of 0.25, 0.5, 1, 2.5% of coconut oil to salads which consist of 20% spinach, 35% tomatoes, 25% carrots, 10% lettuce, 10% yellow peppers, compared to salad which obtained 0.25% to 1% of coconut oil did not affect the micellarization of lycopene, but the micellarization increased after adding of coconut oil at dosage 2.5%. Colle et al. (2012) in an *in vitro* experiment, reported that lycopene bioaccessibility increased after addition of 0, 1, 2, 5, to 10% coconut oil. Moreover, Colle et al. (2012) expressed the addition of 2% olive oil with high long-chain fatty acid to tomato pulp, caused the highest lycopene bioaccessibility but this bioaccessibility was decreased in higher levels of olive oil. Beside that, this experiment discribes that lycopene retention in tomato waste by emulsification with coconut oil better than emulsification with palm oil. Because coconut oil contain less unsaturated fatty acids than palm oil (table 1). The oils with high unsaturated fatty acid highly susceptible to oxidation, forming highly reactive radicals, resulting lycopene depletion. Because lycopene prevent unsaturated fatty acids from being oxidised and resulting in less

absorption of available lycopene in intestinal tarct (Clark et al., 2000).

Lycopene retention in this study was obtained 40.19% (data not displayed) at the control treatment (no oil addition), and 41.85% to 61.74% for the treatment combination the type and dosage of oil in steaming tomato waste powder. Lycopene retention in this study showed a good influence to increase the absorption of lycopene in poultry digestive tract, because of both types of oil (palm and coconut oils) were supporting factor for lycopene absorption in poultry digestive tract. The increasing of lycopene absorption in the digestive tract of poultry due to lycopene is a part of carotenoid. These carotenoid compounds are soluble in oil (Reboul, 2019). Both of oils and lycopene in the digestive tract of poultry would be absorbed in the micelles form. Micelles consist of carotenoid compounds, monoglycerides, and free fatty acids produced by hydrolysis of lipids in digestive tract (Yonekura and Nagoa, 2007). Monoglycerides, and free fatty acids produced by hydrolysis of oil would increase the formation of micelles, consequently lycopene will be more entered into the micelle and absorbed by the body through enterocytes. So that presence lipid would increase lycopene absorption (Hof et al., 2000; Yonekura and Nagao, 2007; Colle et al., 2012; Trujillo and Mc-Clements, 2016).

Carotenoid absorption such as β-carotene was reported by Williams et al. (1998) ranged from 2 to 50%. The absorption is possible from food ingredients and independently from outside of foods. Jain (1999) stated that from 143 µg of lycopene given daily to mice, just 105µg (73%) could absorb. According to Hof et al. (2000) and Trujillo and Mc-Clements (2016) absorption in foods containing carotenoids can be improved by processing. The heat processing will disrupt cell wall structure in plant thus can be released charotenoids from chromoplasts and increase their bioaccessibility (Trujillo and Mc-Clements, 2016) as well as it can change trans form to cis form (Basaran et al., 2017). In present study, lycopene retention have high value compared to previous studies, which was because of tomato waste powder that was a product from steam processing. Steaming of tomato wastes for 12 minutes at 98°C supported releasing of lycopene from tomato matrix and undergo isomerization of lycopene from trans form to cis form. The release of lycopene from tomato matrix will increase the availability of lycopene, while the cis form of lycopene will be absorbed more easily than to trans lycopene form. According to Boileau et al. (1999) and Knockaert et al. (2012), the cis isomer of lycopene was more easily absorbed than the trans lycopene isomer in the digestive tract.

Nitrogen retention and CF digestibility of steaming tomato waste powder increased after adding both of palm and coconut oils compared with nitrogen retention and CF digestibility at tomato waste powder without oil addition (data not displayed). It means, the addition of both type of oils to waste powder of steaming tomato affected the digestion rate in the digestive tract of poultry. The reason can be related to the actions of protease enzyme for hydrolyze protein and lipase enzyme to hydrolize lipid from waste powder of steaming tomato in intestinal lumen of animals. The addition fat to animal feed reduced the passage rate of the digesta in the gastrointestinal tract, and allowed better absorption of all nutrients presented in the feed (Baiao and Lara, 2005; Latshaw, 2008; Rizal, 2013). The addition of 0.25% of oil to steaming tomato waste powder resulted in nitrogen retention 44.55%, and increased to 54.28% when dosage of oil increased to 0.5%, however in the dosage of oil at 0.75, 1, and 1.25 %, nitrogen retention decreased to 41.28%, 36.79% and 30.65% respectively. The decreasing of nitrogen retention caused by addition of higher dosages of oil (more than 0.5%), can be explained by accumulation of lipids in gastrointestinal, which causes low hydrolyze of triglycerides contained in the oil via lipase enzymes, and it mixed with CF contained in the steaming tomatoes waste, so triglycerides would be taken out of the digestive tract and nitrogen retention will be reduced.

The CF content in tomato waste powder was high (12%), which increase the viscosity of the digestive tract, and accelerate the rate of feed in the digestive tract. Therefore the digestibility of CF decreased when the dosage of both oils (palm and coconut oils) increased from 0.5% to 0.75, 1, and 1.25%. Paudel (2013) reported the effect of different types of oil like soybean oil (4%), and the combination of 2% rapeseed oil with 2% flaxseed oil on fat digestibility in broiler chickens and reported that the volume and viscosity of feed in the small intestine increased and the excreta from jejunum had much more liquid and so this might be a reason of low digestion. The range of CF digestibility in present study was from 9.29% to 36.26%. This finding was lower than the report of Mahata et al. (2018) that indicated CF digestibility of unboiled and boiled tomatoes added to broiler diet was from 37.61% to 51.28% respectively.

CONCLUSION

The addition of coconut oil in steaming tomato waste powder increased lycopene and nitrogen retention, and crude fiber digestibility higher than the addition of palm oil to steaming tomato waste powder in the broiler. The best level for lycopene and nitrogen retention, and crude fiber digestibility in broiler was for group that added 0.5% coconut oil to the diet.

DECLARATIONS

Acknowledgments

This Research was funded by the Ministry of Research Technology and Higher Education of the Republic of Indonesia through PMDSU programe. We are very Grateful to Ministry of Research Technology, and Higher Education of the Republic of Indonesia and Rector of Universitas ANDALAS for their support in this program. We are very grateful to the Minister of Research, Technology and Higher Education of the Republic of Indonesia for support and finance through PMDSU No: 1387 / E4. 2015 and Rector of Universitas ANDALAS for their support in this program.

Competing interests

The authors declare that they have no competing interests.

Author's contribution

Handayani were involved in the data collecting, statistical analysis and drafting of the manuscript. Wizna, Suliansyah, Rizal, and Mahata read and approved the final manuscript.

Consent to publish

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

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 196-203, December 25, 2019

Research Paper, PII: S2322455X1900025-9
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DOI: https://dx.doi.org/10.36380/jwpr.2019.24

The Effect of *Aspergillus fumigatus* Infection on Antibody Immune Response to Newcastle Disease Virus in Broiler Chickens

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Received: 22 Oct. 2019
Accepted: 29 Nov. 2019

ABSTRACT

Aspergillus fumigatus infection might predispose birds to other respiratory infections with other pathogens such as Newcastle Disease Virus (NDV). This study aimed to investigate the incidence of *Aspergillus fumigatus* in commercial farms and its histopathological effects on respiratory organs and to evaluate the immunosuppressive effect of aspergillosis on NDV vaccinated birds. *Aspergillus fumigatus* was isolated from feedstuff and broilers in farms with respiratory manifestation. Twenty NDV-vaccinated broiler chickens of 10 days old were experimentally infected by feeding on feedstuff contaminated with *Aspergillus fumigatus*. Twenty vaccinated broilers but not fed the contaminated diet were used as the control group. Clinical signs, histopathological changes, NDV antibody levels in infected birds were recorded. Clinically, infected birds showed respiratory distress, dyspnea, gasping, ruffled feathers, green watery diarrhea, anorexia, lethargy, and unilateral drooping of wing. Histopathological changes were observed as disseminated granulomatous foci in the affected lungs, with caseous necrosis and leukocytes infiltration. The antibody immune response against NDV significantly reduced in infected birds compared with that of non-infected broilers. It is concluded, that *Aspergillus fumigatus* infection suppresses the immune responses and predisposes the broilers to other microbial infections, leading to considerable economic losses in the poultry industry.

Key words: *Aspergillus fumigatus*, Immunosuppression, NDV vaccine

INTRODUCTION

The genus Aspergillus belongs to a filamentous fungal group with wide dispersion in the environment and consists of approximately 200 species (Dagenais and Keller, 2009). Aspergillosis is a respiratory infection caused by fungi of the *Aspergillus* genus, in which *Aspergillus fumigatus* is the primary species responsible for infections in birds and mammals (Souza and Degernes, 2005). Regarding the types of aspergillosis, *A. fumigatus* is the most pathogenic organism causing brooder pneumonia in young chickens, turkeys, and waterfowl (Akan et al., 2002; Beytut et al., 2004; Cortes et al., 2005). Important economic losses are caused in young chicks and turkey infected with *A. fumigatus* (Lupo et al., 2010). Aspergillosis in birds could be initiated following the inhalation of large numbers of spores over a short period of time or chronic exposure to low levels of spores that are widely distributed in nature, thus birds frequently contact them through contaminated feed or litter, resulting in mycotic lesions in the respiratory tract (Arnè et al., 2011; Queiroz et al., 2013). The fungus spores are too small and can be able to reach the lungs and air sacs. The air sacs are usually the primary infection sites since inhaled air reaches the posterior thoracic and abdominal air sacs prior to contacting epithelial surfaces in the lungs (Nardoni et al., 2006). Although *A. fumigatus* is a ubiquitous and opportunistic fungal pathogen causing respiratory tract infections, other organs can also be involved. In addition, aspergillosis can affect many kinds of birds including chickens, turkeys, geese, ducks, quails, ostriches, parrots, canaries, pigeons, penguins and starlings (Cacciuttolo et al., 2009). Stress is a major predisposing factor for the development of the disease (Copetti et al., 2004). Inhalation of a large number of fungal spores is associated with a severe fungal infection that acted as a stress factor and suppresses immune responses due to the production of toxins such as gliotoxin (Gumussoy et al., 2004; Yokota et al., 2004). Aspergillosis occurs in commercial farms in two pathologic forms: acute outbreaks with high morbidity and high mortality found in newly hatched birds in particular and also in free-ranging fowls or psittacines under the poor sanitary or ventilation conditions following

inhalation of the spores that lead to brooder pneumonia, whereas the chronic outbreaks found in adult birds (Tomee and Kauffman, 2000; Beernaert et al., 2010) and characterized by diffuse focal lesions in the viscera (Kunkle, 2003).

Newcastle disease is one of the most important viral diseases that cause severe economic losses in the poultry industry worldwide (MacLachlan and Dubovi, 2011). The causative agent of this disease is known as Newcastle Disease Virus (NDV) or Avian paramyxovirus 1. This virus has been recently classified into the genus *Avian orthoavulavirus 1* within the subfamily *Avulavirinae*, this subfamily includes three genera, *Orthoavulavirus* genus, *Paraavulavirus* genus and *Metaavulavirus* genus in the family *Paramyxoviridae* (Dimitrov et al., 2019).

It has been reported that humoral immune responses are superior in protecting birds against virulent NDV isolates compared to cell-mediated immunity (Reynolds and Maraqa, 2000). Contrary, another report declared that resistance of birds to challenge with virulent strains after vaccination was associated with T memory cell stimulation, resulting in active lymphocytes that combat the disease (Miller and Koch, 2013).

Therefore, the current study investigated the incidence of aspergillosis in commercial farms based on microbiological isolation of *A. fumigatus* to study histopathological changes in organs of infected birds. In addition, this study assessed the immunosuppressive effect of *Aspergillus fumigatus on* NDV antibody titer in vaccinated broilers.

MATERIALS AND METHODS

Ethical approval

Scientific Ethical Committee approved the research and give the ethical number (Vet 14 Medicine November 2018 A and K).

Sample collection

During the periods from November 2018 to February 2019, 10 broiler flocks at Diyala province with heavy respiratory infections and high mortality rates were evaluated in this study. Infected chickens showed signs of gasping, dyspnea, green watery diarrhea, and anorexia. Randomly selected diseased birds were subjected to postmortem examination and swab samples were collected from infected organs and transferred to the microbiology laboratory for culturing. Samples were collected from poultry ration spoiled due to humidity and subjected to mycological examination.

Samples processing

All samples collected from birds and rations were cultured on Sabouraud Dextrose Agar (SDA) (Oxoid, Hampshire, UK) and incubated at room temperature for mycological examination. The fungal hyphae were stained with lactophenol cotton blue and examined with 40X lens under the light microscope (Baron and Finegold, 1990).

Aflatoxin testing

Feedstuff samples were collected from farms with brooder pneumonia and subjected to the Veterinary Laboratory of Baquba Veterinary Hospital, Diyala Province for aflatoxin testing.

Broilers vaccination

Forty one-day-old broiler chicks were supplied by local hatcheries. The birds were divided into two groups (A and B) of 20 birds each. They were separated completely from each other and fed with standard grower dry ration. At the age of 10 days, maternal antibodies against NDV were detected by ELISA kit (Synbiotic, USA) in nine birds randomly selected from each group. Thereafter, both groups of birds were vaccinated with the NDV vaccine (Clone 30, The Netherlands) delivered via drinking water at 10 days old.

Experimental infection

One day post-vaccination, 11-day-old broilers of group A fed with dry concentrated ration, whereas, group B fed with fungal contaminated feedstuff. The broilers in both groups were observed daily until the appearance of clinical signs.

Post-infection sampling

When respiratory clinical signs appeared, the post-vaccination antibody level against NDV was estimated in both groups by collecting blood samples without anticoagulant. Serum samples were separated and anti-NDV antibody titers were determined by the same abovementioned ELISA kit according to the manufacturer's instruction. Birds that showed severe respiratory signs were subjected to postmortem inspection for gross and histopathological examination. Samples from internal organs displaying lesions were immediately fixed with the 10% neutral buffered formalin to avoid the alteration of the tissues through autolysis, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin following trimming and blocking in paraffin. Then 5–6 µm thick cross-sections were prepared and

stained with Hematoxylin-Eosin (H&E) according to the recommended procedure (Luna, 1968). Stained sections were viewed under an Olympus image analysis microscope and recorded (Olympic, Japan).

Statistical analysis

All data were analyzed using the SPSS software version 24. The values less than 0.05 were considered significant.

RESULTS

The overall incidence of aspergillosis in 10 flocks was 70-90%. These farms were poorly ventilated, overcrowded, and humid. Clinical signs and postmortem findings of affected birds clearly indicated aspergillosis. The flock history including location of the flock, population of birds per flock, age of birds, morbidity, and mortality rates are presented in table 1.

Table 1. Morbidity and mortality rates among broilers of commercial farms suspected of aspergillosis in different area of Diyala province, Iraq

Farm number	Location	Age of birds (day)	Number of bird	Morbidity rate	Mortality rate
1	Baquba	3	60004	80%	50%
2	Al-Mokdadia	4	6000	90%	70%
3	Kanan	6	5000	90%	60%
4	Baladrose	5	7000	80%	50%
5	Al-Kales	4	6000	76%	50%
6	BaqubaH	10	7000	90%	70%
7	Baquba	4	7000	90%	70%
8	Baquba	5	7000	80%	50%
9	Al-Mansoria	3	5000	90%	70%
10	Al-Mokdadia	4	6000	90%	70%

Gross lesions

The post-mortem examination of chicks showed numerous small white-yellowish caseous nodules (<1mm in diameter) and large roughly spherical granulomatous nodules (>2 cm) located in the lung (Figure 1). Similar lesions were observed on the surface of other tissues such as kidney, thoracic wall, and abdominal serosa. Nodules observed in the lungs and air sacs corresponded to acute aspergillosis lesions.

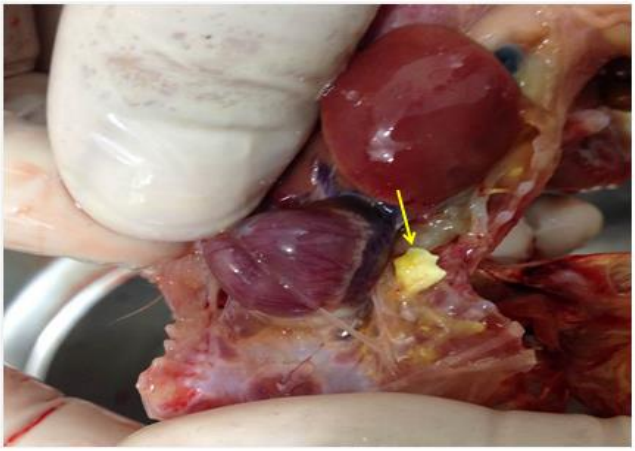

Figure 1. Creamy to yellow color nodules throughout the lung (arrow)

Culturing of swab samples from both infected organs and ration showed fungal growth obtained on day 5 post-culture, with velvety gray to white colonies at first then turned to dark green (Figure 2 A). Fungal samples picked up from colonies and stained with Lactophenol cotton blue, microscopically showed sterigmata, septate hyphae bearing conidiophore vesicle, conidiophore, and chains of pigmented conidia. The conidiophore vesicle was incompletely covered with flask-shaped sterigmata (Figure 2B).

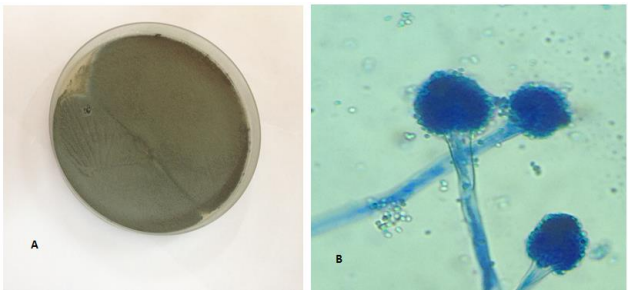

Figure 2. A: Grey-whitish color colony of *Aspergillus fumigatus* appeared on Sabaroud dextrose agar. **B:** Conidiophores with sphere-shaped or semispherical conidia (Lactophenol cotton blue staining).

Aflatoxin detection

The report of Baquba Veterinary Hospital indicated that the contaminated feed contained 43.7 ppb, which is considered a very high level of aflatoxin in poultry feedstuff.

Experimentally infected broiler chickens

Experimentally infected birds in group B showed the first clinical signs 3 days post-infection (PI) that developed quickly within the next 3 days when two birds died. General clinical signs observed in these birds were similar to those from clinical cases of 10 flocks, which included respiratory distress, dyspnea, gasping (Figure 3), ruffled feathers, green watery diarrhea, anorexia, stunting growth, lethargy, and unilateral drooping of the wing due to infection of the thoracic air sac.

Eight days post-infection, six birds died due to severe respiratory signs, and were subjected to postmortem examination and showed similar gross lesions mentioned in clinical cases of 10 flocks studied. These lesions were observed in the kidney, gizzard, and air sac (Figures 4, 5, 6 and 7). Histopathological examination of tissue samples from infected lungs showed disseminated granulomatous foci in tissue of the lung and air sacs. The center of the granulomatous foci contained caseous necrosis and necrotic cellular debris surrounded by rims of heterophils, lymphocytes, macrophages, and multinucleated giant cells. Also, vascular congestion was observed (Figure 8 A and B).

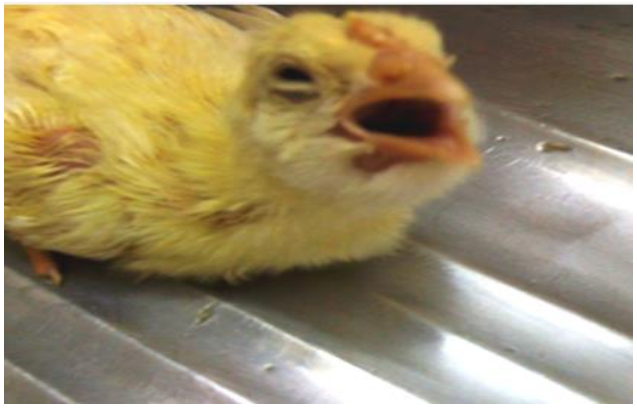

Figure 3. The signs of gasping and dyspnea in broiler chicken

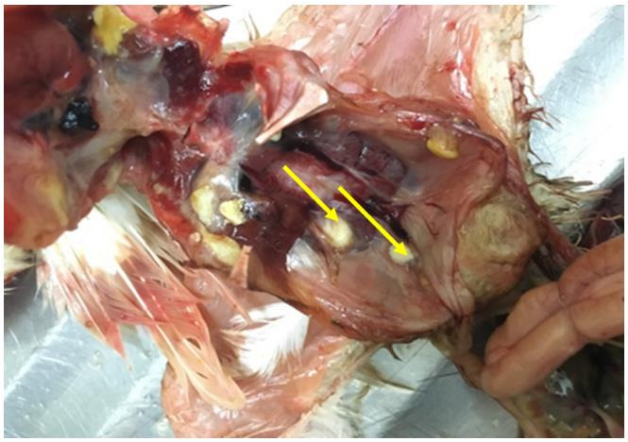

Figure 4. White-yellowish caseous nodules on the kidney.

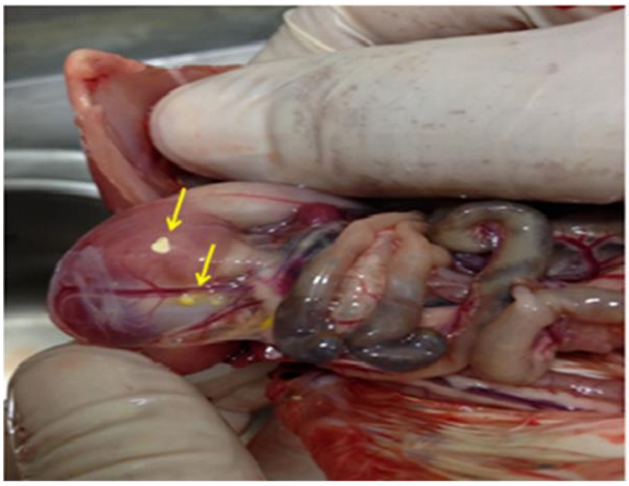

Figure 5. White-yellowish caseous nodules on the outer surface of the gizzard.

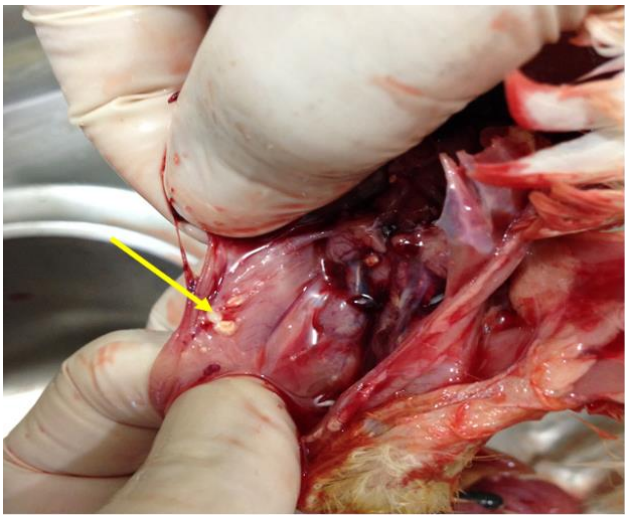

Figure 6. White-yellowish caseous nodules on the wall of thoracic air sac.

Figure 7. White-yellowish caseous nodules on the wall of the abdominal air sac.

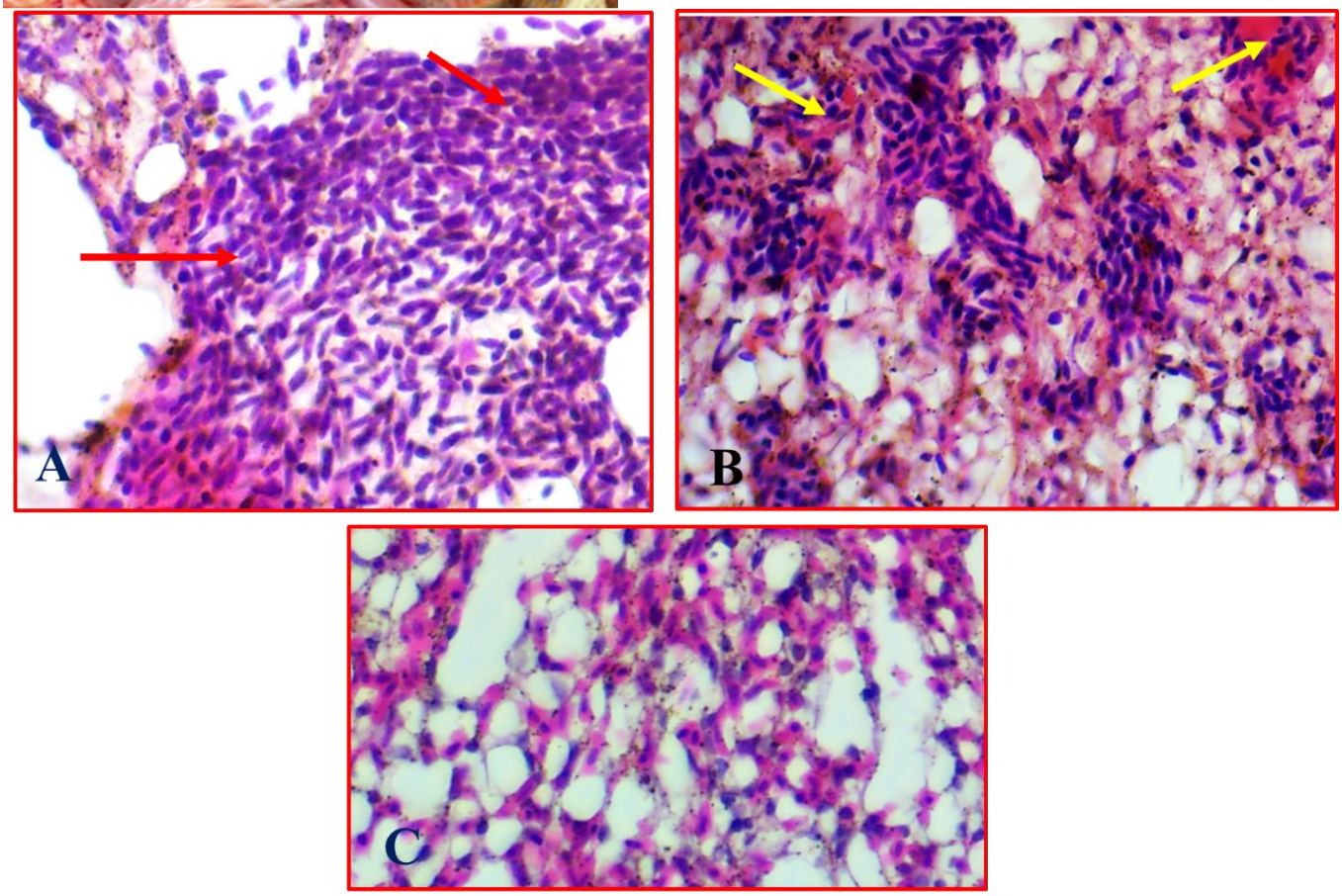

Figure 8. Lung tissues of broiler chickens with experimental aspergillosis. Granulomatous lesions with severe mononuclear cell aggregation in pulmonary tissues consist mainly of macrophage (A, red arrow) as well as vascular congestion (B, yellow arrow) comparing to normal lung (C). **(**40X, H&E stain)

Aspergillus fumigatus was isolated from samples collected from affected organs of experimentally infected birds. Two birds died 3 days PI and were followed by the death of six birds 8 days PI. While 14 days PI another 10 birds died and only two birds survived. Broiler chicks of group A did not show any clinical signs and the birds survived until the end of the experiment.

Newcastle disease antibody levels

The mean anti-NDV maternal antibodies for two groups were 2298.11 AU (Table 2). The first respiratory signs appeared in birds of group B at 13 days of age. Blood samples were collected at 16 days of age from broilers of groups A and B to estimate anti-NDV

antibodies post-vaccination. The mean antibody level was 10325.5 AU in group A and 3083.33 AU in group B (Table 2).

The comparison of maternal and post-vaccination antibody titers showed that the level of antibodies in the uninfected group was significantly ($p \leq 0.05$) higher than that of the fungi-infected group as well as the maternal antibodies.

Table 2. Determination maternal and post-vaccination antibody titers against Newcastle disease virus in fungi-infected and uninfected broiler chickens, Iraq

Ab response to NDV	No. of sampled birds/ total birds	Age of bird at time of blood collection	Mean Ab	GMT	SD	%CV
Maternal Ab against NDV in group A and B	18/40	10 days old	2298.11[b]	1954	268.273	36.79
Ab titer against NDV 6 days PV in group A	18/20	16 days old	10325.50[a]	6165	4314.163	34.53
Ab titer against NDV 6 days PV in group B	18/20	16 days old	3083.33[b]	1663	2172.733	53.20

Group A: NDV vaccinated and uninfected broiler chickens. Group B: NDV vaccinated and fungi-infected broiler chickens. NDV: Newcastle disease virus, Ab: antibodies, AU: antibody unit, PV: post-vaccination, GMT: Geometric mean, SD: Standard deviation, CV: Coefficient of variation. No: number. Different superscript letters in a column indicate a significant difference ($p \leq 0.05$).

DISCUSSION

In the present study, attempts were made to investigate the incidence of avian aspergillosis in commercial broiler farms at Diyala province. A similar incidence was described by Kapetanov et al. (2011) who reported the incidence of aspergillosis was more than 70-90% in most countries with tropical climate. Bhattacharya (2003) declared that aspergillosis occurs sporadically in wild birds but commonly in commercial farms.

Many species of *Aspergillus* including *A. nidulus*, *A. flavus*, *A. niger* can cause aspergillosis, but the most predominant cases are due to *A. fumigatus* which has very small spores in comparison to the spores of other *Aspergillus* fungi (Joseph, 2000; Beernaert et al., 2010; Tell et al., 2019).

The major clinical signs observed in both clinically and experimentally infected birds were moderate to severe respiratory distress, dyspnea and gasping, which are similar to those described by other researchers (Bhattacharya, 2003; Musa et al., 2014).

In the present study, remarkable lesions in postmortem examination were similar to those described by previous study (Musa et al., 2014) and clearly indicated aspergillosis, whereas Olson (1969) mentioned that diagnosis of *Aspergillus* in chickens based on clinical signs and postmortem findings is difficult because signs exhibited are non-specific and may be confused with other bird's infection.

The histopathological findings of the present study were in agreement with the other studies that revealed lesions in birds were commonly confined to lungs and air sacs and also reported the presence of caseous necrotic mass surrounded by inflammatory cells in nodular lesions (Yokota et al., 2004; Charlton et al., 2008).

In the present study, all samples cultured on SDA media were positive for *A. fumigatus* on the basis of colony characteristics (white colonies, at first, which turned dark green later). Ustimenko (1982) reported that the pure culture of *A. fumigatus* can be obtained from white to green mold growth on the walls of caseous thickened lungs and air sacs.

Broiler chicks infected with *Aspergillus fumigatus* had a lower level of anti-NDV antibodies than that in uninfected birds, indicating the infection with *A. fumigatus* led to the suppression of humoral immune responses to NDV. These results are consistent with the finding of many studies. Bellocchio et al. (2005) reported that *A. fumigatus* induced immune response of Th2 lymphocytes that had a role in immunosuppression, resulting in increased susceptibility to the infection and reduced survival. In addition, gliotoxin, a metabolite of *A. fumigatus* has the ability to suppress immune responses and cause apoptosis in primary and secondary lymphoid organs (Watanabe et al., 2003; Arné et al., 2011; Fouad et al., 2019). The concentration of gliotoxin in tissues of turkeys with airsacculitis was found to be 70 µg/g (Richard et al., 1996a), and the death of peripheral lymphocytes of those infected turkeys was attributed to high concentration of gliotoxin in their blood (Richard et al., 1994b; Arias et al., 2018).

Celik et al. (2000) reported impairment in the activity of T lymphocytes and macrophages phagocytosis in broilers affected by aflatoxin accumulation. Furthermore, Fontaine et al. (2011) found a new immunosuppressive metabolite of *A. fumigatus* composed of polysaccharide known as galactosaminogalactan. Its

immunosuppressive activity promotes fungus development. Therefore, immune suppression due to fungal infection might predispose birds to other fungal, bacterial and viral infections (Javed et al., 2005).

CONCLUSION

Aspergillosis and aflatoxins suppress immune responses that may facilitate the infection of broilers with other microbial diseases. Further studies are needed to address factors associated with immunosuppression of infected birds in poultry farms.

DECLARATION

Acknowledgment

The authors would like to acknowledge the College of Veterinary Medicine University of Diyala for logistic supports. The authors also acknowledge the staff of molecular biology laboratory for timely help with guidance and support.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors contributed equally to this study and confirmed the final form of article.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 204-210, December 25, 2019

Research Paper, PII: S2322455X1900026-9
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DOI: https://dx.doi.org/10.36380/jwpr.2019.25

Effect of Using Solar Energy and Different Ventilation Rate on Production in Poultry Houses

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Received: 24 Oct. 2019
Accepted: 01 Dec. 2019

ABSTRACT

The main purpose of the present study was to find an alternative source for traditional energy to provide the energy requirements in the poultry industry. The present study was conducted in four poultry houses with different heating systems (solar and conventional) and ventilation rates located in El-Sharkia Governorate, Egypt, during June and July 2018. In this study, it was found that productivity increased by increasing the ventilation rate, where productivity reached 2.3 kg when using a solar heating system with a ventilation rate every two minutes. Productivity decreased in poultry houses with a conventional heating system and was 2 kg in ventilation rate every 2 minutes, and 1.8 kg in the ventilation rate every four minutes. The level of ammonia was also reduced with the ventilation rate every two minutes. Concentrations of ammonia ranged from 22 ppm at ventilation rate every two minutes to 28 ppm at the ventilation rate every four minutes. In addition, solar energy provided good levels of thermal requirements. It was demonstrated that solar energy as an alternative source to the conventional energy, is very efficient and can be applied on a large scale when combined with conventional electricity as a light source and within specified limits.

Key words: Energy balance, Poultry production, Solar heating system, Ventilation

INTRODUCTION

Following climate changes, high temperatures in summer and extreme cold in winter, as well as rising costs of conventional energy, there is increasing interest in renewable energy sources, especially solar energy among industrial and academic sectors.

High environmental temperature incur huge economic losses on the poultry industry due to reduced growth and laying performance of birds, and also cause concern about poultry welfare (El-Kholy et al., 2017).

Poultry are usually raised in barns, with short growth cycles suitable for the enclosed barn environment in the energy-intensive process. Broiler farms rely on ventilation and heating of barn to ensure the well-being and development of birds. Therefore, ventilation and heating control is the largest energy demand for chicken farms (Hamilton et al., 2016).

Poultry needs temperatures ranged from 26 to 35 ˚C and humidity levels between 60 to 75%. The ventilation system is required for the disposal of polluting substances such as carbon dioxide produced by respiration and other gases emitted from poultry waste. Heat is an important factor affecting the quality of production and animal health. Recently, providing the optimal temperature for poultry houses required too much energy due to the remarkable changes in the environment and climate. High energy expense has led to higher overall costs of production in the poultry industry (Beker et al., 2004). Beker et al. (2004) found that ammonia in poultry houses lowers performance and may increase disease susceptibility. It has been suggested that ammonia should not exceed 25 ppm in poultry houses. Ventilation is important to remove moisture, heat, organisms, ammonia and hydrogen sulfide. Ventilation also replenishes oxygen consumed by birds and gas brooders used to heat poultry building. Energy savings have become increasingly important due to climate change and rising energy prices. Nowadays, feed is the largest portion of the cost of poultry production (Tike, 2010). Fuel and electricity costs are still quite low compared with the cost of feed, however, it is expected that their share of total costs increases in the future.

The main objective of the present study was to use solar energy in poultry farms.

MATERIALS AND METHODS

The experiments were performed in four identical poultry farms in El-Sharkia governorate, Egypt from June to July 2018. The altitude of the study area is 18 m and geographic coordinates of area are latitude 30°10′to 31°19′N, longitude 32° 15′ E . One-hundred cobb500 chicks were used in every house. Chicks were delivered at the age of one-day-old and weight of 40–45 g and slaughtered after 35 days growing period. The chicks were high in vitality. The diet consisted of a starter (3050 Kcal /kg), grower (3050 Kcal/kg) and finisher feed (2900 Kcal /kg).

Poultry house

The houses were designed in a closed system. Modern poultry houses were made of or bricks and concrete with industrial ventilation and cooling system. Broiler house is heated mainly using renewable energy. The size of the houses was approximately 4 m in length, 2.5 m in width and 3 m in height (Figure 1), with a capacity of 100 birds. The average bird density was 10 bird/m^2. In present experiments, plastic pan feeders with a diameter of 30 cm (70 birds per pan), and bell drinker (50 birds/bell) were used.

Ventilation system

The farms were supplied with fans that drain the air from inside the farm to the outside. The fan engine model was 3165-00 manufactured in China (power: 90 watts, rotation speed: 300 rpm, engine: 220-240 volts, 50-60 Hz) and fan dimensions were 50 × 50 cm. The fan was installed from the side. The capacity of each fan was 4000 m^3 of air/h with a flow rate of 4 m^3 air/h/kg of live weight in summer. The fan had shutters that open when the fan was running and close by gravity when it was idle.

Cooling system

In this experiment, direct evaporative cooling systems were used. This system consisted of the cooling pad (dimensions: 60 × 50 cm; thickness: 10 cm) and fan. The system has a 100 watts pump that pumped water from the water tank to the pad. There were also lines to return water to the tank.

The heating system and power sources

The traditional heating system in poultry houses used two sources of energy. The first source was fossil fuels such as petroleum or natural gas and the second source was electricity and an electric meter connected with each experimental unit to measure energy consumption. In the house, the electric heater has a power of up to 1500 W. As shown in figure 2, the solar heating system consisted of the three connected subsystems including solar thermal collector, thermal storage system and heat distribution system.

The dimensions of the typical flat-plate solar collector were 80 × 120 cm, with a thickness of 10 cm. Absorber surface plate (dimensions: 95 × 95 cm) was painted with black color for increasing solar energy absorption. The absorber plate was welded with 12 horizontal copper pipes of 7 mm diameter (Thermal conductivity coefficient of 400 W/m. K), insulated body by fiberglass (Thermal conductivity coefficient of 0.04 W/m. K). In this design, the main energy storage system used was sensible heat storage. The heating system was equipped with a capacity of 300 L water and a 300 W pump. In the design of the solar heating unit, calculations were performed based on the maximum thermal requirements of the farm. The hot fluid inlet of the tank was fed by the water circulating from the thermal solar collector. The solar heating unit was manufactured locally from iron pipes connected to each other in an entry line and a water return line of 100 × 100 cm.

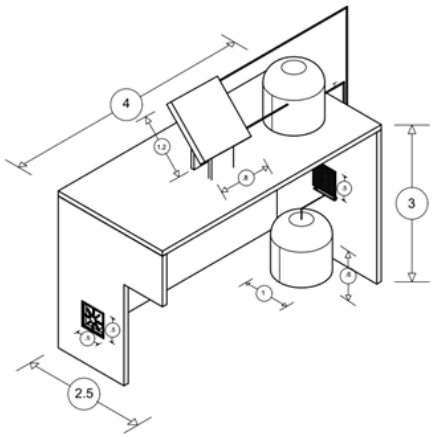

Figure 1. Poultry house with a solar collector

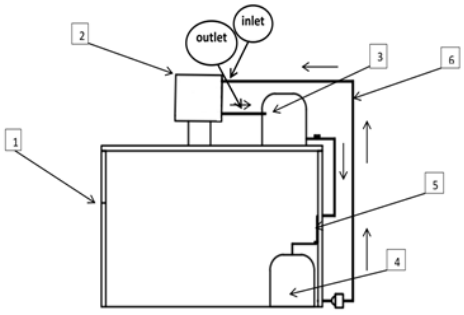

Figure 2. Poultry house components. 1. Door; 2. Solar collector; 3. Water storage tank; 4. Receiver tank; 5. Cooling pad; 6. Return line.

Experimental conditions

House 1 was working with solar heating unit and ventilated every 2 min. House 2 was working with a traditional heating system and ventilated every 2 min. House 3 was equipped with solar heating unit and ventilated every 4 min and house 4 was working with a traditional heating system and ventilation rate of 4 min.

Thermal performance analysis of solar water heating system

The basic parameter to consider was the efficiency of solar thermal collectors, defined as the ratio of the useful heat energy delivered to the solar energy flux incident on the collector aperture. Under steady-state conditions, the performance analysis can be measured and determined using the system analysis described Kalogirou (2004) as follows:

Solar energy available was obtained via the following equation: $Q = R\ A_c$

Where Q is solar energy available, R is solar radiation falling on collector surface (W/m^2) and A_c is the surface area of the solar collector (m^2).

Absorbed solar radiation (Q_α) was calculated using the following equation:

$$Q_a = R\ A_c\ (\alpha\ \tau)$$

$$\alpha = \alpha_{max} - 0.00476 \exp[0.040\ (\theta - 35)]$$

$$\tau = \tau_{max} - 0.00437 \exp[0.0936\ (\theta - 30)]$$

Where ατ is optical efficiency of the solar collector (decimal), α is the effective absorption of the absorber surface (decimal), τ is the effectiveness of permeability of covered glass (decimal) and Θ is the solar incident angle on the tilted surface (degree).

Heat removal factor (F_R) is a quantity that relates actual useful energy gain of a collector to useful gain and was calculated by the following equation:

$$F_R = \frac{m\ C_P\ (T_{fo} - T_{fi})}{Ac\ [R(\alpha\tau) - Uo\ (T_{fi} - T_a)]}$$

Where m is the mass flow rate of fluid (kg/s), C_p is specific heat of water (J. kg^{-1}. K^{-1}), T_{fo} is outlet water temperature (°K), T_{fi} is internal water temperature (°K), T_α is ambient air temperature (°K) and Uo is overall heat transfer coefficient (W/m^2. °K).

Useful heat gain (Q_u) is defined as the maximum obtained energy of the solar collector when the water passed through the pipes of collector and determined using the follow equation:

$$Q_u = F_R\ [Q_a - U_o\ A_c\ (T_{fi} - T_a)]$$

Solar heat loss (Q_L) is identified as the difference between absorbed solar energy and useful heat gain to the storage, as follow:

$$Q_L = Q_a - Q_u$$

The loss in the heat of the solar heater (Q_L) can be also calculated by the following equation:

$$Q_L = A_c\ U_o\ (T_P - T_a)$$

Where T_p is the mean temperature of the absorber plate (°K).

Thermal efficiency of solar collector (η_o) was calculated by the following equation:

$$\eta_o = \frac{Qu}{Q} \times 100$$

The stored solar energy (Q_s) in the storage tank can be determined by using the following equation:

$$Q_s = M_w\ C_p\ (T_{ke} - T_{kb}) / \partial\tau$$

Where M_W is water mass (kg), T_{ke} is the water temperature in a storage tank at end of day (°K), T_{kb} is the water temperature in the storage tank at beginning of day (°K), $\partial\tau$ is the time interval during which water circulates within the system (s).

The storage system efficiency (η_s) can be calculated as: $\eta_s = \frac{Qs}{Q_u} \times 100$

Calculation of ventilation rate

The air change rates were calculated by the following equation:

QA = live Wight × number of chicken × minimum ventilation rate (m^3/h per Kg live weight) (Arbor Acres, 2009). Where QA is the standard quantity of air to be removed

Calculation of energy consumption

Ventilation heat loss

The heat loss caused by ventilation could be calculated by the following equation:

$P = c_i\ \rho_i\ q_v\ (T_{in} - T_{out})$P = ventilation heat loss

c_i = air specific heat capacity, 1,0 (kJ/kg.K)

ρ_i = air density;

q_V = ventilation volume flow;

T_{in} = indoor temperature (K)

T_{out} = outdoor temperature (K)

Poultry heat production

The broilers also produce heat, the sensible heat production was calculated by CIGR2002 method (Mannfros and Hautala, 2011), using following equation:

$$p_{sens} = 10.62\ M^{0.75}\ [0.61\ (1 + 0.020\ (20 - T)) - 2.28.10^{-4}\ .T^2$$

Where P is heat power (W), M is broiler mass (kg) and T is temperature (°C).

Measurements and determinations

Air velocity Meter (TM-411/412/413/414) was used in the poultry houses to measure different macroclimate variables such as the dry-bulb air temperature (ventilated thermistor), wind speed, wet-bulb air temperature and air relative humidity (hygrometer).

RESULTS AND DISCUSSION

Climate conditions

One of the external climate factors affecting the internal environment of a poultry house is solar radiation. The maximum solar radiation value in June was 1119.2 W/m^2 in the afternoon, the radiation value at the beginning of the day was 220 W/m^2 and at the end of the day, the radiation intensity was 110 W/m^2 at 6 p.m. (Figure 3).

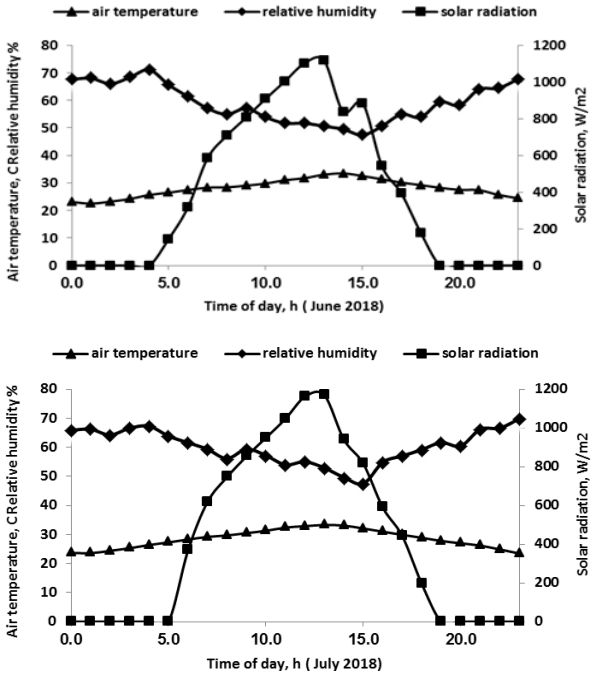

Figure 3. Distribution of solar radiation, air temperature, and relative humidity during June and July 2018 in El-Sharkia Governorate, Egypt.

According to the Egyptian Meteorological Authority, in July, the maximum value of solar radiation at midday hours (12-1 p.m.) was 1166.6 W/m^2 and the lowest radiation value was found at sunset (6 p.m.) and the radiation value was 190 W/m^2. The external temperature and relative humidity are important factors affecting the poultry house environment. The outdoor temperature affected heat levels inside the houses. The maximum value of the external temperature in June was 33 °C in the afternoon, with a relative humidity of 47.5%. The lowest temperature recorded in June was 23.1 °C (Figure 3). The maximum value of the temperature in July was 33.5 °C and relative humidity was 49.2%. The lowest temperature recorded in July was 23.3 °C (Figure 3).

Internal microclimate

When measuring the internal temperature and relative humidity, each house should reach the temperature and humidity level appropriate to the need of chicks at different ages. The temperature should gradually decrease as the bird ages. It was found when using a ventilation rate every two minutes, the temperature gradually decreased so that it did not affect chicks' health and thermal burden. As shown in figure 4, the highest temperature value (33°C) was recorded when the ventilation rate was every 2 minutes using the conventional system. The temperature continued to decrease to the end of life. In the traditional system, the temperature reached 27.1 °C while in the solar systems reached 26.3 °C which was closer to the thermal requirement of the herd according to Arbor Acres (2015).

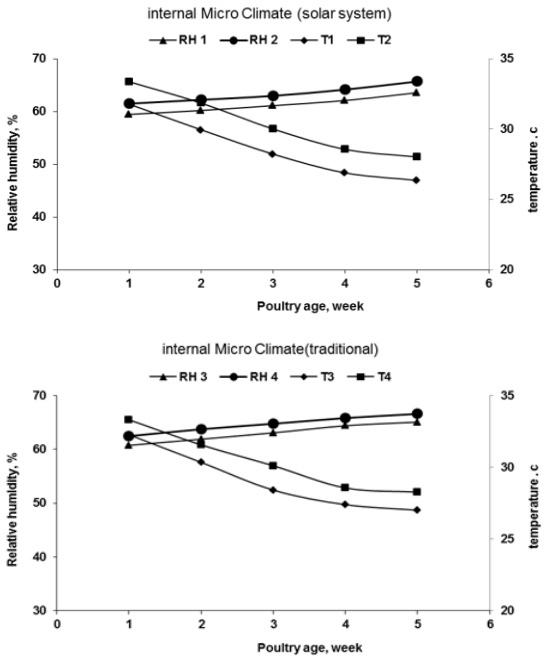

Figure 4. Internal microclimate in experimental poultry houses with the different heating systems located in El-Sharkia Governorate, Egypt, during June and July 2018. **T1**: temperature in house 1, **T2**: temperature in house 2, **T3**: temperature in house 3, **T4**: temperature in house 4. **RH 1**: relative humidity in house 1, **RH 2**: relative humidity in house 2, **RH 3**: relative humidity in house 3, **RH 4**: relative humidity in house 4. **House 1**: solar heating system and ventilation rate every 2 min. **House 2**: traditional heating system and ventilation rate every 2 min. **House 3**: solar heating system and ventilation rate every 4 min. **House 4**: traditional heating system and ventilation rate every 4 min.

In poultry houses with ventilation rate every four minutes with two heating systems, the highest temperature was recorded in house 4 (33.5 °C). The temperature continued to decrease to the end of life. In the traditional system, it reached 28.1 °C and in the solar systems reached 27.5 °C. The relative humidity was affected by quantity of air. When the ventilation rate was every 2 minutes, humidity in house 1 was lower than house 2 due to high quantity of air.

Ammonia gas emission

Ammonia level is an indicator of the quality of the ventilation process. The increased ammonia in the house environment adversely affects poultry health and also causes problems for farm workers (Zong et al., 2014). Figure 5 presents ammonia levels within the houses.

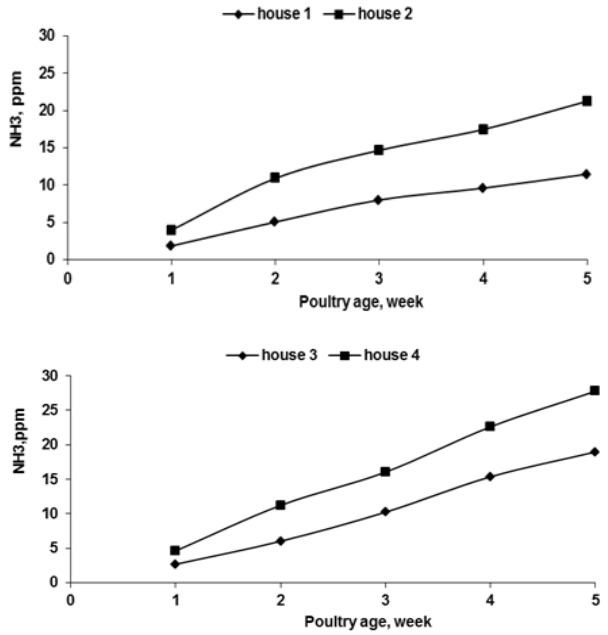

Figure 5. Internal ammonia gas emissions in experimental poultry houses with the different heating systems located in El-Sharkia Governorate, Egypt. **House 1**: solar heating system and ventilation rate every 2 min. **House 2**: traditional heating system and ventilation rate every 2 min. **House 3**: solar heating system and ventilation rate every 4 min. **House 4**: traditional heating system and ventilation rate every 4 min.

The ammonia levels at the beginning of life were low and gradually increased with age. When using a ventilation rate every two minutes, the average ammonia concentration in the first week was 1.8 ppm. Traditional systems led to more ammonia emissions. Emission of ammonia in the solar systems reached 15 ppm while in the conventional system it reached 20 ppm. Ammonia concentration with increasing age showed an upward trend in all farms. When the ventilation rate was every four minutes, the level of ammonia in the first week reached to 4 ppm. At the end of the rearing cycle, this rate increased up to 20-23 ppm. This finding was similar to the results of Alloui et al. (2013) and Beker et al. (2004). Ammonia concentration in the house with conventional systems and ventilation rate every two minutes, reached higher than 22 ppm while the level of ammonia was 28 ppm in ventilation rate every four minutes.

Ventilation rate and heat loss

The energy loss was averaged over the duration of ventilation and delivery walls. The energy loss obtained in each experimental house ranged from 0 to 0.5 kWh/kg and this finding was similar to Rajaniemi (2012). In the summer with the increase of poultry age, the heat in the poultry house was less than outside. The energy loss recorded in each farm ranged from 0 to 0.7 KWh per kg of live weight that was similar to the results reported by Rajaniemi (2012). As showed in figure 6, the loss of thermal energy was 0.56 kWh for the first week.

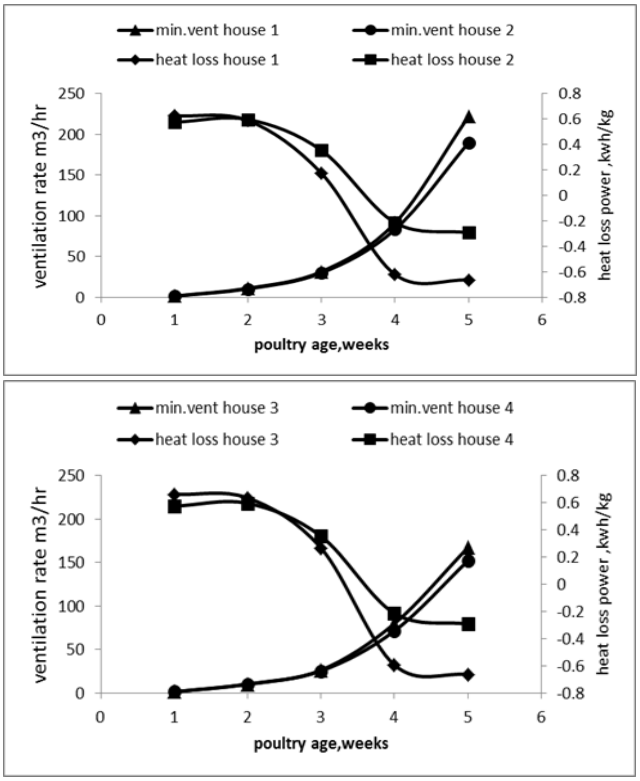

Figure 6. Minimum ventilation rate and heat loss in experimental poultry houses with different heating systems located in El-Sharkia Governorate, Egypt. Min. vent: minimum ventilation rate. **House 1**: solar heating system and ventilation rate every 2 min. **House 2**: traditional heating system and ventilation rate every 2 min. **House 3**: solar heating system and ventilation rate every 4 min. **House 4**: traditional heating system and ventilation rate every 4 min.

Poultry weight

The meat produced from poultry houses at the end of the rearing period varied and provided evidence from the quality of the production process. The highest average weight of meat produced by a chicken was 2.35 kg when using solar heating system with a ventilation rate every two minutes. It was expected that the high ventilation rate, low levels of ammonia and other toxic gases lead to increased weight. In the summer, ventilation rate every two minutes provided optimum heat and heat elimination resulting in increased production.

In the solar heating system and ventilation rate every four minutes, the weight dropped slightly and reached 2 kg. This decline was due to several reasons; the first reason was the low conversion coefficient caused by pathogens as well as the increase in the average temperature. The second was high thermal pressure in the summer season leading to low productivity.

Solar heating system

As showed in table 1, the efficiency of the solar heating system was 71.2% in June. Heat loss in the solar heating system was 9.2 kWh/day, the maximum stored energy value was up to 20.6 kWh/day and the storage efficiency of the solar system was 90.7%. The total efficiency of the solar heating system in July was 70.9%, but the energy loss was 9.8 kWh/day and the energy consumption was 23.8 kWh/day. The solar energy was stored in tanks as hot water (60 °C) with storage efficient up to 91.2% and the losses were low that was consistent with results reported by Kalogirou (2004).

Figure 7. The mean Slaughter weight of birds in experimental poultry houses with different heating systems located in El-Sharkia Governorate, Egypt. **House 1**: solar heating system and ventilation rate every 2 min. **House 2**: traditional heating system and ventilation rate every 2 min. **House 3**: solar heating system and ventilation rate every 4 min. **House 4**: traditional heating system and ventilation rate every 4 min.

Table 1. Thermal performance analysis of solar heating system

Month	Q	Q_a	F_R	Q_u	Q_L	η	Q_s	η_s
	KWh/day	KWh/day	decimal	KWh/day	KWh/day	%	KWh/day	%
June	38.158	31.90009	0.86	22.71624	9.183847	71.15067	20.6	90.68402
July	40.10232	33.52554	0.87	23.76746	9.758085	70.8911	21.68	91.21716

Q: solar energy available, Q_a: Absorbed solar radiation, F_R: Heat removal factor, Q_u: Useful heat gain, Q_L: Solar heat loss, η: Thermal efficiency of solar collector, Q_s: Solar energy stored, η_s: Storage system efficiency

CONCLUSION

This study concluded that the use of mechanical ventilation is better than conventional and a ventilation rate every two minutes is highly beneficial. The use of solar energy with electricity as a mixed heating system produces good results.

DECLARATION

Authors' contributions

Al authors contributed equally to this study and confirmed the final edition of article.

Competing interests

The authors declare that they have no competing interests

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 211-216, December 25, 2019

Research Paper, PII: S2322455X1900027-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.26

The Effect of *Bacillus subtilis* Inoculum Doses and Fermentation Time on Enzyme Activity of Fermented Palm Kernel Cake

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Received: 29 Oct. 2019
Accepted: 06 Dec. 2019

ABSTRACT

Palm kernel cake (PKC) was by-product of palm oil industry and it had potential to be one of the poultry ration ingredient However, its utilization for poultry was still limited because of the β-mannan in PKC. In order to increase PKC utilization in poultry ration, fermentation process was done to remodeled β mannan by using *Bacillus subtilis.* This research conducted a study on the effect of *Bacillus subtilis* inoculum dose and fermentation time to increase the enzyme activity of FPKC by using CRD with 3 × 3 factorial and 3 replications. Factor A was 3 doses of inoculum *Bacillus subtilis:* 3%, 5%, and 7%. Factor B was fermentation times which contained: (1) 2 days, (2) 4 days, and (3) 6 days. Parameters used were enzyme activity of mannanase, protease, and cellulase in FPKC. Significant interaction was seen between inoculum doses of *Bacillus subtilis* and fermentation time. There was also a significant interaction on each of the inoculums dose of *Bacillus subtilis* and fermentation time on all of the enzyme activity. This study concluded FPKC with *Bacillus subtilis* of 7% inoculums doses and 6 days fermentation time indicate the best result as seen from 24.27 U/ml of mannanase activity, 10.27 U/ml of protease activity, 17.13 U/ml of cellulose activity of fermented PKC.

Key words: *Bacillus subtilis*, Enzyme activity, Fermentation time, Inoculum doses, Palm Kernel Cake

INTRODUCTION

The availability of feed ingredients is still a common problem experienced by farmers, because of its insufficient availability. Various ways have been tried in order to availability become sufficient. One of the efforts to overcome that problem is by using plantation waste as a cheaper alternative feed ingredient, while not competing with human needs, such as palm kernel cake.

Indonesia as the largest palm oil producer in the world, has produced 45 million tons of palm oil/year (DGP, 2016). In West Sumatra, oil palm plantations have an area of 399,120 hectares, producing 1,145,432 tons palm oil per year (Central Bureau of Statistics, 2015). Each of fresh palm bunches produces 5% of palm kernel and it also produces 45-46% of palm kernel cake or 2.0-2.5% by weight of palm bunches. Nutritional content of palm kernel cake is 17.31% crude protein, 7.14% crude fat, 27.62% crude fiber, 0.27% Ca, 0.94% P and 48.4 ppm Cu (Mirnawati et al., 2018).

The utilization of palm kernel cake in broiler rations is limited to only 10% (Rizal, 2000). Low utilization of palm kernel cake in poultry rations is due to its high mannan content. As stated by Daud and Jarvis (1993). The 56.4% of crude fiber content in palm kernel cake is in a form of β-mannan. However, poultry do not have β-mannan hydrolysis enzyme in their bodies. In addition to the high crude fiber content in palm kernel cake, its low protein and amino acid digestibility also caused low utilization of palm kernel cake. As well as, Tafsin (2007) found that the low utilization of palm kernel meal in poultry rations is caused by the high content of crude fiber, low digestibility of proteins and amino acids. Fermentation is one of the methods to improve the quality of the palm kernel cake.

Fermentation is a change in chemical material of feed ingredients because of the enzymes produced by microorganisms or existed in these feed ingredients (Buckleet al., 1987; Rizal et al., 2013). Fermented palm kernel cake was done with the help of mannanolytic fungis, for it can produce mannanase enzyme that hydrolyzes mannan. Mirnawati et al. (2017) studied palm kernel cake fermentation using mannanolytic fungis such as *Sclerotium rolfsii, Eupenicillium javanicum* and *Aspergillus niger*. The *Sclerotium rolfsii* has a higher ability among the three fungis to produce enzyme

compared to *Eupenicillium javanicum* and *Aspergillus niger*. *Sclerotium rolfsii* has mannanase enzyme activity of 24.58 U / ml and cellulose activity of 21.89 U / ml and can improve the nutritional quality of palm kernel cake, as seen from 23.66% of crude protein, 16.72% of Crude fiber, 0.22% of crude lipid, 0.75% of Calcium, 0.85% of Phosphor, 57.16% of nitrogen retention, and 25511 kcal/kg metabolizable energy of palm kernel cake. Although there has been an increase in the nutritional content of fermented palm kernel cake with *Sclerotium rolfsii*, its utilization in broiler rations is only up to 25% (Mirnawati et al., 2018).

Fermentation with the help of fungi takes longer time than microbes. According to Fardiaz (1992), microbes as an inoculum requires less time than fungi in the fermentation process, which is about 1-2 days, because the division time is faster. In addition, there are also mannanolytic microbes which are *Bacillus subtilis* WY34 (Jiang et al., 2006). Hooge (2003) added that *Bacillus subtilis* can produce several enzymes such as protease, β-mannanase and several enzymes that are useful in helping digestion so that it is easier to digest. *Bacillus subtilis* is known to be capable of producing cellulase when placed in an environment containing cellulose. *Bacillus subtilis* can produce protease, α-amylase, and renin (Darwis and Sukara, 1990). Many factors including dose of inoculum and length of fermentation need to be considered following the fermentation process. The application of the right inoculums dose will provide opportunities for rapid growth and development of microbes when more doses of inoculum used, the fermentation process occurs faster, thus more substrates changed. Furthermore, the longer fermentation period lasts, the more substances that are remodeled (Fardiaz, 1992 and Mirnawati et al., 2013).

Therefore, it is necessary to know the optimal dose and duration of palm kernel cake fermentation with *Sclerotium rolfsii* to produce maximum enzyme activity to increase the nutritional value of palm kernel cake.

MATERIALS AND METHODS

The materials used in the study were: palm kernel cake, fine bran, *Bacillus subtilis* InaCC B289, media (NA/ Nutrient Agar), distilled water, buffer solution, and chemicals for analysis of mannanase activity, cellulase and protease. The tools were autoclaved and analytical scales were made in Japan. Incubators, spectrophotometers, and shakers waterbath are made in Jerman. Centrifuges, erlenmeyer, pH meters were made in China.

This research was conducted using experimental methods using a completely randomized design (CRD), a factorial pattern consisting of 2 treatment factors, namely factors A and B with three replications (Steel and Torrie, 1991). Factor A is the dose of inoculum, namely: A1 = 3%, A2 = 5% and A3 = 7%. Factor B is the duration of fermentation, namely: B1 = two days, B2 = four days and B3 = six days. Measured variables were the activity of Mannanase, cellulase and Protease.

Fermentation

Fermentation carried out with a ratio of 80.20 palm kernel meal and bran with 60% moisture content. Materials autoclaved for 15 minutes at 121^{o} C, Chilled at room temperature then *Bacillus subtilis* inoculated on ingredients. After inoculation, *Bacillus subtilis* then fermented in the incubator.

Enzyme extract

10 g of the fermented sample, then soak with 90 ml of 0.05 M pH buffer phosphate. Leave it in the shaker incubator at 100 rpm for 30 minutes. After that, strain with filter paper and take the filtrate. The filtrate obtained was centrifuged with 10,000 rpm, temperature of -4^{o}C for 15 minutes. Take the supernatant and the enzyme activity will be analyzed.

Enzyme activity measurement

- *Mannanase and protease by N-Somogyi Nelson Method* Mannanase and protease by N-Somogyi Nelson Method

One ml of crude enzyme substrate was added to one ml of manan substrate (0.5 g/ml manan plus 10 ml of phosphate buffer). Incubate for 30 minutes at temperature (40 cellulases and 60 mannanases) in the waterbath shaker. Take one ml extract of the enzyme that has been incubated, and then add the Nelson AB solution. Heat in boiling water for 20 minutes, after that cool and add one ml of phosphomolibdat solution and seven ml of distilled water measure with a Uv-Vis spectrophotometer wavelength 575 nm.

- *Protease* Protease

The crude extract of the extracted enzyme determined by its proteolytic activity based on Cupp and Enyard (2008). One ml of crude enzyme extract was added to the casein substrate 0.65% (0.65 g casein in 100 ml Pospat buffer 0.05 M pH 7.5). The reaction mixture was incubated at 37° C for 10 minutes. Termination of the reaction was carried out by adding five ml of 110 ml TCA reagent, and re-incubating at 37° C for 30 minutes. Two ml of filtrate was separated by centrifugation at 10000 rpm for 10 minutes. Five ml of Na2CO3 and one ml of Folin

Ciocalteau reagent were added to the filtrate and incubated at 37° C for 30 minutes. The absorbance of the mixture was measured using a UV-vis spectrophotometer at a wavelength of 660 nm.

Data analysis

Datas obtained were processed statistically by analysis of variance of a completely b randomized design (CRD) with factorial patterns 3x3 with tree replicates. Differences between treatments test by using Duncan multiple range test (DMRT) (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Treatment effect on mannanase activity

The mean mannanase activity of fermented PKC with *Bacillus subtilis* was illustrated in table 1. Table 1 showed that there was a tendency of increase in mannanase activity along with the addition of the inoculum dose. Increasing doses of inoculum caused the higher ~~the~~ activity of mannanase on 2 days, 4 days and 6 days of fermentation time. Furthermore, the longer fermentation time increases mannanase activity, at the inoculum dose of 3%, 5% and 7%. The above data could be concluded that the 7% inoculum and 6-days fermentation time (A3B3) provided the highest mannanase activity.

The high mannanase activity of A3B3 (6.27 U/ml) was concluded by high doses of inoculum and the longer time of fermentation given to microorganisms allows it to grow and develop rapidly, thus increased mannanase activity. Consistent with Mirnawati et al. (2017) that reported giving the more doses of inoculum caused faster fermentation process, because of the high doses of inoculum that enables increase of enzyme activity and microbial growth in the substrate. Fardiaz (1992) found that the slow pace of fermentation greatly determined the amount of enzymes produced in the media. The longer the fermentation time occurs, the more substrate will be degraded by enzymes produced by microbes. The low mannanase activity in the A1B1 treatment (15.49 U/ml) was caused by smaller dose of inoculum, which is at 3% and short duration of fermentation (2 days). This caused the slow growth of microbes and low activity of enzyme, however with an increase in the inoculum dose of 7%, the growth of microbes will be better. So, the dose of 7% inoculum is the optimum doses for the growth of *Bacillus subtilis*. In accordance with Darwis et al. (1995) at the beginning of fermentation, enzyme activity was very low and it will increase along with the increasing fermentation time. The enzyme activity follows the growth pattern, starts from the phase of adaptation, exponential, stationary and death phase.

Table 1. Mean mannanase activity fermented palm kernel cake with *Bacillus subtilis*

Factor A (Inoculum Dose)	Factor B (Fermentation time)			Mean
	B1 (2 days)	B2 (4 days)	B3 (6 days)	
A1	5.49^{aAB}	5.62^{aB}	5.52^{aC}	5.55
A2	5.60^{bB}	5.66^{bAB}	5.94^{aB}	5.73
A3	5.80^{bA}	5.81^{bA}	6.27^{aA}	5.96
Mean	5.63	5.70	5.91	--

Note: Different uppercase letters in different columns and small letters on the same row showed very significant different ($P < 0.05$). Mean mananase activity is in U/ml.

Treatment effect on cellulase activity

The mean cellulase activity of fermented PKC with *Bacillus subtilis* is shown in table 2. There was an increase of cellulase activity as seen from table 2, along with the addition of the inoculum doses. Cellulase activity was increased at fermentation times (2 days, 4 days, and 6 days), proofing that more length of fermentation will increase cellulase activity. Inoculum doses influenced by cellulase activity, evidenced by an increased in cellulase activity with more inoculum doses of 3% 5% and 7% inoculum doses. From the above data, it can be concluded that the 7% inoculum dose and 6 days fermentation time (A3B3) provides the highest cellulose activity at 16.11 U/ml.

The high cellulose activity of A3B3 treatment at 16.11 U/ml caused by the increasing doses of inoculum and the length of fermentation given which allows the rapid growth and development of microorganisms, so the cellulose activity will be increased. In accordance with the opinion of Mirnawati et al. (2013) that higher dose of inoculum provided better environment for microbial growth causing faster fermentation process while also increasing the enzyme In addition, fermentation time is also one of the determinant factor, where longer fermentation time will cause more remodeled substrate that produced by enzyme of microbes (Mirnawati et al. 2012).

The low cellulose activity in the A1B2 treatment (5.49 U/ml) caused by low doses of inoculum at 3% and 4 days of fermentation time. At this treatment, microbes tend to growth slowly and the enzyme activity will be lower. However, the increase in the inoculum dose of 7% was causing microbes to grow better, thus 7% inoculum dose is recommended as the optimal dose for the growth of *Bacillus subtilis* which is in accordance with Zulfatus et al. (2008) that enzyme activity was obtained at post exponential (stationary) time after the 4th day of fermentation. At the incubation period, it was shown that the cellulose enzyme worked optimally in hydrolyzing the substrate, namely cellulose found in palm kernel cake, into glucose.

Treatment effect on protease activity

The mean protease activity of fermented PKC with *Bacillus subtilis* is shown in table 3. There is a tendency of an increase in protease activity as presented in table 3, along with the supplementation of inoculum dose. The increased doses of inoculum come with the increased protease activity, both at fermentation times (2 days, 4 days and 6 days). Longer time of fermentation also increases the protease activity, at the inoculum dose of 3%, 5% and 7%.It can be concluded from the above data that the 7% inoculum dose and 6 days of fermentation time (A3B3) shows the highest protease activity at 10.27 U/ml. The highest protease activity is shown from A3B3 treatment, it was caused by the increased dose of inoculum and the longer fermentation time, so microorganisms may grow and develop more while also increasing protease activity. In accordance with the opinion of Musaalbakri et al. (2005), the number of inoculums has effect on increasing cell concentration gradually because it increases microbial growth. Thus by increasing the inoculum, the microbes will produce more protease enzymes. Dada et al. (2009) also found that high doses of inoculum added would increase the metabolic compounds produced, while higher inoculum concentrations were also inefficient when the fermentation process was carried out. The longer the fermentation time, the higher the enzyme activity produced. According to Dwidjoseputro (2010) that there is an incubation time in producing metabolic compounds of each bacterium according to the growth phase of each bacterium.

The low protease activity in the A1B3 treatment at 5.36 U/ml was caused by a small inoculum dose at 3%, resulting in low microbial growth and enzyme activity. The increasing dose of inoculums will provide a better environment for the growth of microbes, thus 7% inoculum is the optimum dose for the growth of *Bacillus subtilis*. This is in accordance with the statement of Belma et al. (2000) that reported incubation time affects the cell growth process which occurs through cell division. That process will increase the living cells and the rate of growth in its culture. During the incubation period, it was shown that protease enzymes performed optimally in hydrolyzing the substrate, namely the protein found in palm kernel cake into amino acids and protein quality increases (Mirnawati et al., 2019).

Table 2. Mean cellulase activity of fermented palm kernel cake with *Bacillus subtilis*.

Factor A (Inoculum Dose)	Factor B (Fermentation time)			Mean
	B1 (2 days)	B2 (4 days)	B3 (6 days)	
A1	12.34^{bB}	12.33^{bC}	13.15^{aC}	12.608
A2	12.44^{cB}	13.37^{bB}	14.97^{aB}	13.596
A3	13.38^{cA}	14.47^{bA}	16.11^{aA}	14.654
Mean	12.72	13.392	14.744	--

Note: Different uppercase letters in different columns and small letters on the same row show very significant different .($P<0.05$). Mean cellulose activity is in U/ml

Table 3. Mean protease activity (U/ml) of fermented palm kernel cake with *Bacillus subtilis.*

Factor A (Inoculum Dose)	Factor B (Fermentation time)			Mean
	B1 (2 days)	B2 (4 days)	B3 (6 days)	
A1 (3%)	5.85^{aB}	6.34^{aB}	5.36^{aC}	6.38
A2 (5%)	6.95^{bA}	6.68^{abB}	8.31^{aB}	7.31
A3 (7%)	7.21^{bAB}	7.61^{bA}	10.27^{aA}	8.36
Mean	6.67	6.87	7.98	

Note: Different uppercase letters in different columns and small letters on the same row show very significant different (P<0.05). Mean Protease activity is in U/ml.

CONCLUSION

The high inoculum doses and the longer fermentation time can increase enzyme activity in fermented palm karnel cake. The 7% inoculum doses and 6-days fermentation time provided optimum results as indicated in mannanase activity (6.27 U/ml), cellulose activity (16.11 U/ml) and protease activity (10.27 U/ml).

DECLARATIONS

Acknowledgements

This study was financially supported by funds provided by BOPTN of Andalas University, number 42/UN.16.17/PP.RGB/LPPM/2018, dated April, 23rd 2018.

Author's contribution Mirnawati conducted the research, and prepared data. Gita Ciptaan did the field research and Ferawati performed statistical analysis. All authors checked and confirmed the final form of article

Competing interests

The authors have declared that no competing interest exists.

Consent to publish

All authors gave their informed consentprior to their inclusion in the study.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 217-223, December 25, 2019

Review Paper, PII: S2322455X1900028-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.27

Probiotics and Poultry Gut Microflora

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Received: 28 Oct. 2019
Accepted: 09 Dec. 2019

ABSTRACT

Poultry production is presently the most effective animal production industry and provides an excellent source of protein production worldwide. The poultry gastrointestinal microbiota includes commensal, mutualistic and pathogenic microbes. The relationship between host and gut microbiota can affect the balance of mutualism and pathogenicity. The imbalanced gut microflora caused by the incidence of disease, hygiene conditions, diet, management practices, and environmental stress affects the survival and productivity of chicken. Maintenance of the gut microbial composition is possible through the regulation of the gastrointestinal microbiota by suppressing the growth of pathogens. For many years, antibiotic growth promoters have been used to manage these problems. Nowadays, because of the emergence of antibiotic-resistant bacteria, other alternatives are being sought. Supplementation of probiotics as feed additives is considered to enhance chicken productivitity and to protect the gut from pathogen colonization and help to tolerate environmental stress. The goal of the present article was to review the poultry gastrointestinal microflora and probiotics role in the health and growth of poultry. In addition, this article focused on probiotic microorganisms and their potential characteristics.

Key words: Gastrointestinal microbiota, Poultry, Probiotics

INTRODUCTION

Poultry production is currently the most efficient animal production system and forms the basis of global protein production (USDA, 2019). The advantage of poultry production depends on the ability of chickens to efficiently convert feed into muscle mass. This makes them an effective system for producing high-quality proteins (Phillippa et al., 2018). According to FAO (2012), poultry refers to the domestic birds including domestic chickens (*Gallus gallus* domesticus), turkeys, ducks, geese, dove, and other domesticated birds that are raised to produce eggs and meat. Among these, chicken production is the most popular worldwide. The interaction between the biochemical functions of the poultry and the intestinal microbiota is involved in extracting energy and nutrients from food. Thus, the selection of beneficial microbiota plays an important role in improving production performance, detoxification, modulation of the immune system and protection against pathogens (Clavijo and Florez, 2018). In the poultry, different organs contribute to the digestion and absorption process of nutrients. Microorganisms present in each organ of the digestive system have independent functions and different taxonomic composition. As a result, gut organs are considered as separate ecosystems for microbes despite the deep interconnection between gut microflora (Wielen et al., 2002).

The microbiota in the poultry gastrointestinal (GI) tract includes commensal, mutualistic and pathogenic microorganisms. The gut microbiota positively influences the GI development, immunological and physiological functions of the gut. In poultry, these microorganisms colonize the GI tract during the early post-hatch period and form a synergistic relationship with the host (Torok et al., 2008). Chicken gut microflora composition changes in relation to the age of chickens, dietary factors, breed, and geographic location. The different factors related to diet, infectious agents, environmental and management conditions negatively affect the balance of poultry gut microbiota, which consequently impairs feed conversion ratio and growth performance (Yegani and Korver, 2008). The balance between pathogenicity and mutualism can be

determined by the relationship between the host and its gut microbiota. Modulation of the GI microbiota by suppressing the growth of pathogens helps to maintain the optimal microbial composition. Hence, the inclusion of antibiotic growth promoters in animal diets improves growth and feed conversion efficiency (Dumonceaux et al. 2006). The emergence of antibiotic-resistant bacteria causes the growing global concerns related to the transmission of these bacteria from animals to humans. This global concern has led to limiting the usage of antibiotics in livestock (Apata, 2012). Therefore, the alternative attention is concentrated on the use of probiotic microorganisms and other products such as enzymes, organic acids, bacteriocins, bacteriophages and nanoparticles that can similarly enhance poultry productivity and produce safe edible products (Mehdi et al. 2018). In addition, following the European Union ban on the use of prophylactic antibiotics in poultry nutrition, scientists currently enforced to seek alternatives to antibiotic growth promoters to produce safe and efficient poultry meat and egg (Saeed et al. 2017).

Microflora in the chicken gastrointestinal tract

The digestive tract of chickens is comprised of the crop, proventriculus, gizzard small and large intestines and ceca (Nasrin et al., 2012). In addition, gut microflora, gut-associated immune tissue, liver, gall bladder, and pancreas are other important components of the digestive system (Dibner and Richards, 2004). The bacteria are the most abundant microbes of the GI tract. Approximately, there are up to 10^{10}-10^{11} bacteria per gram of cecal content. Fungi and protozoa are the other gut inhabitant microbes (Albazaz and Buyukunal, 2014). Archaea which is represented predominantly by methanogenic *Methanobrevibacter* are other microorganisms colonized in chicken gut (Saengkerdsub et al. 2007). The specialized microbial communities in the GI tract perform important digestive functions as feed passes (Oakley et al. 2014). In chicken, the main bacterial activities are found in crop, small intestine, and cecum (Albazaz and Buyukunal, 2014). According to the report of (Youssef et al., 2017) inclusion of probiotics on poultry feed resulted in a numerical reduction in intestinal aerobes and fecal coliforms. Furthermore, all probiotics used significantly reduced total aerobic and staphylococci counts in the carcass meat, with a numerical decline in *E. coli* count. A prolonged feed retention time in the crop is associated with significant degradation of starch and fermentation of lactate mediated by the microbial community with the predominance of various *Lactobacillus* species. Also, *Clostridiaceae* family resides in the crop (Svihus, 2014). The species of *Lactobacillus* and *Clostridiaceae* also are present in the gizzard. However, the existence of pepsin, gastric juices and hydrochloric acid in the gizzard decreases the pH and leads to reduced bacterial populations and fermentation activity (Clavijo and Florez, 2018). In poultry, the lower intestinal tract involves the small intestine, the colon, and two big cecal chambers which are important for the fermentation process (Sekelja et al., 2012). The small intestine is colonized mainly by *Lactobacilli* followed by *Streptococci* and *Enterobacteria*. On the other hand, the caecum is colonized mainly by strict anaerobes and a small number of facultative anaerobes (Cisek and Binek, 2014). The alimentary tract in newly hatched healthy chicken is usually sterile. The development of chicken intestinal microflora depends on their contact with bacteria from the environment within the first days after hatching. Differences in bacteria ingestion from hatching debris, environment, producing facility, feed and water cause variation in the microbial populations (Binek et al., 2000). On the first day of chick's life, the cecal microflora consists mainly of *Enterobacteriaceae*, *Enterococcus* and *Lactobacillus* species. After the second week of age, *Bacteroides* and *Eubacterium* species were established (Borda-Molina et al., 2018). Various species, different individuals of the same species and distinct sections of the GI tract have a different composition of microorganisms. In addition, the gut microflora is unstable over time (Dibner and Richards, 2004).

Impact of poultry gut microorganism on host

The gut is a natural barrier between the host and the intestinal microflora. There are numerous bacterial cell communities and millions of genes in the host. The expression of this amount of genes helps them to perform numerous enzymatic reactions that the host is not able to catalyze. This enables the microflora to influence many aspects of intestinal tract development and to provide metabolic contributions to the host (Yeoman et al., 2012). Generally, the gut microflora has a prominent role in digestion, metabolism, vitamin synthesis, immune stimulation and pathogen exclusion (Amit-Romach et al., 2004). Production of highly specialized hydrolytic enzymes by gut microorganisms allows degradation of complex substrates like non-starch polysaccharides and other indigestible carbohydrates (Sergeant et al., 2014). This hydrolysis allows further fermentation of the feed components by other members of the gut ecosystem that generate short-chain fatty acids, which in turn become accessible to the host as energy and carbon sources.

(Wang et al. 2016). The products and activities of hydrolytic enzymes create an ecosystem that is appropriate for some bacterial genera and hostile to others (Panda et al. 2009). Apart from nonpathogenic microbes, harmful members of the gut microflora may be involved in local or systemic infections, intestinal putrefaction and toxin formation (Yasothai, 2017). Enteric pathogens such as *Escherichia*, *Campylobacter*, *Vibrio*, *Shigella*, *Yersinia*, and *Salmonella* are a major cause of poultry morbidity and mortality throughout the world. Gram-negative enteric pathogens cause diarrhea and fever (Foley et al. 2013).

Probiotic microorganisms

The term probiotic has been defined as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" (Fuller, 1989). Probiotics stimulate the growth of beneficial microorganisms, reduce the number of pathogens, and lower the risk of gastrointestinal diseases (Getachew, 2016). These living microorganisms are nonpathogenic and harmless in nature, that are favorable to the host's health when properly administered through the digestive route (FAO/WHO, 2001). These microorganisms include different species that belong to bacteria, fungi, and yeasts (Chen and Zhu, 2017). Youssef et al. (2017) also reported that probiotics and acidifiers can be used as potential alternatives to antibiotics in broiler diets. Different microbial species or different strains of the same species have different probiotic potential. Specific receptor sites and particular immunological properties are some of the reasons accounting for this difference (Hadisaputro and Harimurti, 2015). Probiotic microorganisms can be isolated from plants, food products, environment, human and animal sources (Hossain et al., 2012). Different studies reported the isolation of potential probiotic strains from the natural poultry gut microflora (Ehrmann et al. 2002, Shin et al. 2008). Competitive elimination of pathogenic microbes, production of antibacterial products (such as bacteriocins and colicins) and immune modulation are the basic mechanisms of probiotics. Live non-pathogenic microbial strains, either single or multi-strain, belonging to the genera *Lactobacillus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Pediococcus*, *Aspergillus*, and *Saccharo-myces* are used in poultry (Dhama et al. 2011).

Role of probiotics in poultry production

The poultry industry is a significant financial activity across the globe. Heavy financial losses occur when birds are subjected to stressful environmental conditions and disease. The emergence of a wide range of antibiotic-resistant bacteria and pathogens are the main limiting factors for the poultry industry productivity (Kabir, 2009). A stable protective flora is established naturally in the poultry gut. Some dietary and environmental factors such as stress, antibiotic treatment, and excessive hygiene influence the stable protective gut microflora (Donaldson et al. 2017). Probiotic supplements are used to reconstitute the natural flora of chicken. Different strains of bacteria capable of surviving and inhabiting in the gut are used as probiotics. However, probiotics can be harmful to immunocompromised populations. The correct dosage of probiotic administration has not yet been established (Getachew, 2016). Several studies have been described the role of different probiotic *Lactobacillus* strains in chicken productivity and health. A study which involved the use of feed supplemented with *Lactobacillus* culture (1 g *Lactobacillus* culture /1 kg feed) in pure Hubbard and pure Shaver chicks from day 21 to 42 resulted in greater weight gain and heat tolerance in comparison to controls (Zulkifli et al. 2010). *Escherichia coli*, different species of *Salmonella enterica* and *Campylobacter jejuni* are the primary pathogens of poultry farming. The administration of *Lactobacillus* probiotics decreases enteric pathogenic microbes through competitive exclusion in the poultry intestinal tract and improves the intestinal well-being (Hadisaputro and Harimurti, 2015). According to Bansal et al. (2011), broiler chicks fed a diet with probiotic yeast gained significantly higher weight than control groups. In addition, dietary intake of Kefir as a probiotics source resulted in a decrease in chicken liver weight (Vahdatpour and Babazadeh, 2016). Diet supplemented to Protexin® probiotic alone or in combination with Fermacto® prebiotic increased growth hormone level and improved growth performance in quails (Nikpiran, 2014). The administration of probiotic supplements via drinking water significantly improved the weight gain in Kenyan indigenous chicken (Atela et al., 2015). The positive effects on weight gain and feed conversion ratio were observed in quails that received synbiotics (Babazadeh et al. 2011). The addition of probiotics to feed increase feed efficiency, growth performance, egg production, meat and egg quality as well as cholesterol level in poultry products (Getachew, 2016; Popova, 2017).

Role of probiotics in protecting poultry gastrointestinal infection

The probiotic microbes have the capacity to inhibit the development of pathogenic microorganisms in the gut of poultry (Getachew, 2016). Supplementation of probiotic

products allows manipulation of the GI microbiota. For example, *Listeria monocytogenes* is one of the pathogenic microbes that affect the poultry GI tract. Administration of multi-strain probiotic containing different *Lactobacillus* species and *Bacillus amyloliquefaciens* prevents the establishment and spread of this bacterium in the GI tract of broiler chickens (Neveling et al. 2017). In another study, the administration of commercial probiotic preparation formulated from different species of *Lactobacillus* and *S. cerevisiae* reduced the stress of *E.coli* K88 infected Hubbard broiler chicks and reduces *E.coli* proliferation in GI tract (Mohamed and Younis, 2018). According to Forkus et al. (2017), the production of the antimicrobial peptide known as Microcin J25 by engineered *E.coli* inhibits colonization of *Salmonella enterica* in the turkey GI tract. *Clostridium perfringens* is a pathogenic microbe that causes necrotic enteritis in poultry and negatively affects poultry health and productivity. Inclusion of *Lactobacillus johnsonii* BS15 to the feed reduces the incidence of necrotic enteritis and damage of villi by necrotic enteritis in Cobb 500 chicks (Wang et al. 2017). Administration of *Lactobacillus plantarum* K KKP 593/p and *Lactobacillus rhamnosus* KKP 825 via feed or drinking water reduce the number of *E.coli* in ROSS 308 broiler chickens (Michalczuk, 2019). According to Shokryazdan et al. (2017), supplementation of chicken feed with a mixture of *L. salivarius* strains improved populations of lactobacilli and decreased harmful bacteria including *E.coli* and total aerobes. Intestinal microbial modification through early probiotic inoculation has a role in improving the weight gain of the host.

At-hatch administration of beneficial strains has different results compared to the natural acquisition of the same strain from the environment (Baldwin et al. 2018). At-hatch administration as compared to natural acquisition improved feed conversion rate, growth performance, resistance to disease, digestion and absorption of nutrients, and carcass quality (Mohan, 2015). Synthesis of the antimicrobial compounds by the probiotic species, such as *Lactobacillus* spp., *Pediococcus acidilactici*, *Lactococcus lactis*, and *Enterococcus faecium* is one mechanism to prevent pathogens colonization. These antimicrobial products including short-chain fatty acids, bacteriocins, hydrogen peroxide, etc. inhibit or kill bacteria such as *Staphylococcus aureus*, *E. coli*, *Clostridium perfringens*, *Salmonella typhimurium*, *Bacillus* spp., *Listeria* spp., *Klebsiella* spp. and *Proteus* spp. by binding to the specific receptors and causing cell damage (Cisek and Binek, 2014).

Characterization of probiotic microbes

The characterization of probiotic is based on the consensus of scientists on some criteria, with particular attention being paid to the ecological origin of the bacteria, tolerance level to the harsh stomach and small intestine environments and capacity to bind to intestinal surfaces (Koenen et al. 2004). In general, microorganisms with potential probiotic advantages share common characteristics. The common requirements or properties of probiotics are discussed below.

General properties of probiotics

During the isolation process of microorganisms for probiotics, different selection criteria should be used as a reference. According to Kosin and Rakshit (2006) and Fuller (1989) some of the conventional criteria that can be applied for the selection of microbial species as probiotics comprise biosafety, the origin of the strain, resistance to GI tract conditions, intestinal adhesion and colonization, antimicrobial activity, stimulation of immune response, survival and stability throughout processing and storing (Khalil et al. 2018). In order to produce the desired effect, the probiotics strains should have a property to grow and survive in the digestive system of the host as they are exposed to a range of stressful conditions in the gut including lower pH, bile and pancreatic juice (Jose et al. 2015). The effects of simulated gastric juice and bile acids on the growth of probiotics are varied among species and strains. Species or strains with the greatest tolerance to acid and bile are excellent targets for the development of probiotics products. In addition, isolates with high tolerance to heat can be selected to produce probiotics (Hossain et al. 2012). Adhesion of the probiotics microbes to the intestinal mucosa is regarded as a precondition for colonization in the GI tract. This capacity to adhere is one of the most significant requirements for the choice of probiotics (Harzallah and Belhadj, 2013). The selection of probiotics also focuses on the safety of microorganisms. Hence, probiotics should be non-pathogenic and have no adverse effect on the host. The probiotic itself or its fermentation products or cell components should not be pathogenic, allergic, mutagenic, and carcinogenic (Harzallah and Belhadj, 2013). As an advantage, the probiotic strains should act as an adjuvant and stimulate the immune system against pathogenic microorganisms (Jose et al. 2015).

One of the safety considerations for selecting a potential probiotic strain is that it does not contain antibiotic resistance genes that can be transferred to the pathogenic microorganisms (Shakoor et al. 2017).

Probiotics microbes may be subjected to antibiotics in the animal gut when antibiotics are used as medicinal products for animal health. As a result, to be effective, the probiotics strains should possess non-transferable resistance which aids them *in vivo* survival (Shakoor et al. 2017). The resistance of probiotics isolates to some antibiotics is considered as an intrinsic property, presenting no safety concerns in feed or food (Khalil et al. 2018). Antagonistic activity of probiotics microorganisms against pathogens is regarded as a characteristic of probiotic to maintain the gut microflora balanced and to keep the gut rid of pathogens. Probiotics inhibit the growth of pathogenic bacteria through the production of nonspecific antimicrobial compounds such as hydrogen peroxide, short-chain fatty acids, and low molecular weight proteins known as bacteriocins and bacteriocin-like inhibitory substances (Torshizi et al., 2008).

Technological characteristics of probiotics

For the wide-scale distribution of probiotics strains, they must be manufactured under industrial conditions. These probiotic microorganisms have to survive and retain their functionality during storage as frozen or freeze-dried cultures. Similarly, their incorporation into foods or feeds should not provide unpleasant flavors or textures (Saarela et al. 2000). Technological evaluations include pH, salt and bile acid tolerance, hydrogen peroxide production, utilization of different carbon sources, enzymatic activities, hemolytic properties, antibiotics sensitivity, antimicrobial activity and *in vitro* adherence properties (Abiodun et al. 2013). Large scale production of probiotics involves a fermentation process. During fermentation reactions, the probiotics strains may be exposed to different temperature conditions. In addition, the storage and transport process of probiotics products should be under the optimum temperature. Thermophilic organisms have the advantage of tolerating higher temperatures during processing and storage. They have a better chance of remaining viable during the drying process required for prolonged storage and thus become distinctly effective products (Kosin and Rakshit, 2006).

Importance of probiotic research

The animal production system has a considerable impact on the nutrition and health status of consumers. Animal intestinal pathogenic microbes including *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria* are the major cause of food contamination and zoonosis. Different methods of animal production are introduced to increase productivity, quality, and safety of animal products, besides protecting animal welfare and the natural environment (Markowiak and Slizewska, 2018). Previously, different medicinal products and antibiotics had been widely utilized to modify the animal gut microflora to enhance productivity and improve animal growth. However, the emergence of drug-resistant microorganisms has been occurred due to the long-term use of antibiotics and other medicinal products which causes a great fear to consumers and it also exerts negative impacts on the environment (Apata, 2012). The usage of probiotics is mentioned as one of the alternatives (Mehdi et al., 2018). Investigation of locally produced probiotics, targeting animals based on their surrounding environment and feed is important to maximize probiotics efficacy and to create market opportunities. Particularly, people in developing countries who do not have access to probiotics and live in different geographical locations will be benefited from locally sourced probiotics (Sybesma et al. 2015).

CONCLUSION

In general, the present review revealed that an effective dose of probiotics can have a dominant role in the improvement of intestinal microflora and production performance. In addition, it can inhibit the development of pathogenic microorganisms in the gut.

DECLARATION

Competing interests

The authors have no competing interests.

Authors' contribution

Kibirnesh Tegenaw designed the review, collected the information, and wrote the manuscript. Dr. Kagira and Prof. Nega collected the information and revised the manuscript.

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 224-232, December 25, 2019

Review Paper, PII: S2322455X1900029-9

DOI: https://dx.doi.org/10.36380/jwpr.2019.28

History and Current Situation of Commercial Ostrich Farming in Mexico

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Received: 30 Oct. 2019
Accepted: 08 Dec. 2019

ABSTRACT

As in many other countries, in Mexico, the ostrich aroused the interest of public and private entities for its broad productive qualities and quality of its products. The objective of the present study was to describe the history of ostrich introduction in Mexico as a kind of commercial interest, from the arrival of the first birds to the current farms. In 1988 the first farm was established, then a series of farms of significant size were appearing, all of them focused their business on the sale of breeding stock, a business that was profitable during the heyday of the specie in the country (1998-2008). The main client was the government that acquired ostriches to distribute them among a large number of new farmers. When the introduction into the activity of government and private individuals was no longer attractive, the prices of the breeders fell and the sector collapsed because the farms were inefficient and the infrastructure and promotion sufficient to position the ostrich products were not produced on the national or export market. In 2016 it was known that about 30 farms remained in the activity, of which 20 were located and provided information for this study. The farms that remained in the activity continued with significant difficulties in terms of their productivity, however, they had managed to mitigate part of the problem by sharing production practices among themselves and going to their counterparts abroad through digital media. On the commercial side, they had managed to develop standardized products using maquiladora companies, and placed them in niche markets that paid for higher prices than those that are paid for conventional substitutes. In the case of ostrich, in Mexico and many other countries, the sector failed because the market demand response was overestimated and the farmers ventured into the activity without adequate knowledge bases, infrastructure, and institutional support. These findings could be referred to many other species of nascent interest.

Key words: Emerging sectors, Exotic poultry, Niche market, Specialty livestock, Organization, Ostrich meat

INTRODUCTION

The commercial use of ostrich had its origin in South Africa, a country that led the production of this species with 60% of the meat, skin and feathers of the world market (Hoffman and Cawthorn, 2014). According to Benson (2012), the first countries that seconded South Africa in ostrich production were the neighbors Namibia, Zimbabwe and Botswana, as well as Australia, Germany, France and Belgium. Then, in a second wave, countries such as the United States, Canada, Spain, Portugal, Italy and Greece were incorporated. Finally, in a third wave, the ostriches arrived in territories of Argentina, Brazil, Peru, Colombia, Venezuela, Chile and Mexico. Ostrich aroused the interest of investors in different parts of the world, due to the productive qualities and integral use of the specie (Brand and Jordaan, 2011; Ghaffari Moghadam, 2016; Abbas et al., 2018), as well as for the nutritional benefits of its meat (Majewska et al., 2009; Polawska et al., 2013; Al-Khalifa and Al-Naser, 2014; Medina and Aguilar, 2014; Abbas et al., 2018). The event that triggered the incorporation of ostrich as a productive specie in various territories was the outbreaks of Bovine Spongiform Encephalopathy in the years 1986 and 2002 in Europe and the United States, respectively, due to the sensory similarity between beef and ostrich meat (Shanawany and Dingle, 1999). In addition, deregulation of live bird exported from South Africa in 1998 was also a fact that facilitated the acquisition of breeding stock by other countries (Pittaway and Van Niekerk, 2015).

Although ostrich production has declined significantly in many countries, the specie remains of great zootechnical interest, because the consumption of its meat is considered an appropriate option for consumers who

liked red meat and were also concerned about their health (Akram et al., 2019). Similarly, interest in the species was reflected by the evolution shown by research in issues related to production efficiency (González-Redondo et al., 2014), improvement in skin quality which is extracted from the ostrich for the manufacture of leather goods (Jordaan et al., 2008) and in the optimization of methods for oil extraction, a product which is known to have important nutritional, cosmetic and pharmaceutical qualities (Ponphaiboon et al., 2018).

Mexico was part of the ostrich heyday. The authority responsible for regulating the management of this species had a record of more than 300 farms between 1991 and 2015. On the other hand, in 2012, the organization in charge the promotion of ostrich meat in Mexico estimated in 800 numbers of farms that incorporated the ostrich, considering all those that were not officially registered. The same promotional agency estimated that in 2016 there were no more than 30 farms whose main activity was the commercial production of ostrich. Under this context, the objective of the present study was to describe the history of the introduction of ostrich in Mexico as a species of commercial interest, from the arrival of the first birds to the current farms.

MATERIALS AND METHODS

The present investigation had a descriptive approach and was carried out based on a sequential mixed design. A mixed design was chosen because it allows generating unique research that answer questions about the complexity nature of the phenomenon being studied, from the point of view of the participants and from the expression of the measurable variables (Williams, 2007). On the other hand, it was sequential because of the study integrated two blocks of analysis, the first was qualitative and referred to the historical description of ostrich production in Mexico, meanwhile, the second was quantitative and described the profile of the farms that until the time of information gathering (Summer, 2016), they were in operation.

The history of commercial ostrich production in Mexico was built from the founding of the first formally registered farm, to the recent events that resulted in the farms that remained active until 2016. Historical description was elaborated according to aims of Laudan et al. (1986), with an analytical construction of social, cultural and economic events that form a present reality. Information on historical events were obtained from interviews with current farm owners, former farmers, former leaders of the organizations now-extinct, officials and former government officials, and marketers.

The description of farms that remained active until 2016 and which had decided to collaborate providing information for the study was based on the descriptive statistics applied to variables and indicators that identify and measure different attributes. For this purpose, interviews were conducted with the owners and the interviewers stayed at least one working day at the farm to observe the internal processes. An observation guide and a semi-structured questionnaire for the interview were implemented as detection instruments. The information collected is related to the following items:

- General characteristics of the farm including name, age, location, scale, characteristics of the owner, importance of income and links with other farms, organizations, and institutions.
- Technical profile and productivity including technical practices implemented during the production process and productivity indicators.
- Products and activities for adding value including prices, presentations, the importance in incomes of each product, facilities for the transformation of products, brands, certifications, destinations and marketing channels, messages and means of promotion.

RESULTS

History of the sector

The story about commercial ostrich production in Mexico began on 1988 when 92 ostriches from South Africa arrived by air the city of Reynosa in the state of Tamaulipas. This was the first official registration farm dedicated to the production and marketing of ostriches and their derivatives. In 1992, this farm had 500 female ostriches and established the first large incubation center for this species in the country, which had an incubator with a capacity of 2600 eggs and a hatcher capable of hatching 380 chickens.

In 1995, there had been the first large distribution of ostriches in Mexico. The state government of Tamaulipas ran a livestock program that bought 600 breeders, and gave one pair (one male and one female) to 300 new farmers, who were largely unsuccessful in commercial management of the specie. A year later, in the state of Sinaloa, the second large farm dedicated to the commercial production of ostriches emerges, which acquired its flock of breeders of the first one that emerged in Tamaulipas. This second farm was distinguished by its

intense work of promoting ostrich meat, through its brand, mainly in the cities of Guadalajara and Monterrey.

In 1997, two more farms were born in the cities of Monterrey and Querétaro. This pair of companies was characterized by being founded by reputable entrepreneurs from sectors other than livestock and the primary sector, reflecting the attractiveness of ostrich production investments at that time. Noteworthy was the fact that these companies were the first to sell the meat they produced in self-service stores. In the same year, Funds Instituted in Relation to Agriculture (FIRA), one of the most important public institutions for financing, training, technical advice and technology transfer of the agricultural sector in Mexico, recognized ostrich as a highly productive specie and publish a manual entitled "The ostrich is a profitable alternative in livestock production in Mexico", so great was FIRA's interest in commercial ostrich production that it implemented a full-cycle demonstration module in the city of Morelia.

A significant number of respondents agreed to point out the year 1998 as the beginning of the ostrich heyday in Mexico, due to the consolidation of the interest shown by various public and private entities for that year. Since then, the responsible agency for regulating the ostrich production units in Mexico was the Secretary of the Environment and Natural Resources (SEMARNAT), through General Directorate of Wildlife (DGVS). Although there were already companies that marketed ostrich products at that time, the industry was generally at a stage of breeding and distribution of breeders and had as its main challenge the adaptation of the specie and domain of artificial incubation.

In 1998, other entrepreneurs emulated the actions of the industry's pioneers and imported ostriches from South Africa, Namibia, Botswana, and Zimbabwe and produced breeders which were bought and distributed by stat governments with similar mechanisms as in Tamaulipas. As could be anticipated, the results were not very different from those obtained in that state.

In 1999, an alliance arose between a farm dismantled in Texas and that moved to the state of Morelos and a farm located in the state of Hidalgo. The alliance gave rise to a brand that managed to sell four tons of ostrich meat monthly in restaurants in Mexico City. Likewise, the alliance distinguished itself by achieving an important opening in media for the promotion of the qualities of ostrich meat.

As far as universities were concerned, it stood out in 2003 that the National Autonomous University of Mexico (UNAM) imported 24 ostriches from the United States to create a practical teaching module; besides, UNAM included the ostrich in the subject of alternative poultry farming. The first farm to obtain the Federal Inspection Type certification was born on 2004 in Jalisco after visiting the UNAM module. This certification in Mexico confirmed that a company has facilities and procedures for slaughtering, cooling and industrialization of meat products that ensured its safety. These standards enabled the company to enter into contracts for the export of ostrich meat to Japan.

In 2004, with the resources of the Secretary of Agriculture, Council for the Promotion of Ostrich Meat (COMEPA) was created, an entity whose main task was to position ostrich meat in Mexican homes and markets. In addition to COMEPA, there were other organizations whose main function was to keep track of the inventories of the associated farmers. The Mexican Association of Ostrich Farmers was one of the most important associations of national coverage, however, there were many other state and regional associations.

The ostrich heyday which began in Mexico in 1998, lasted until 2008. During this period, the sector was promoted by SEMARNAT, FIRA and different state governments. So far, SEMARNAT had been the regulator of ostrich management but had never provided subsidies, because the ostrich was considered an exotic specie in Mexico and SEMARNAT programs focused on the conservation and use of endemic species. FIRA contributed significantly to the promotion of the specie and its products and training the new farmers. The subsidies for ostrich farmers came mainly from state funds, so that the lobbying of large enterprises in their respective federal states was essential to develop programs that distributed assets such as breeders, pens and incubators.

From 2009, the activity decreased. Given the poor results obtained by the great majority of the new producers, public funds for the promotion of the specie disappeared, and with them, a large part of the organizations that sourced resources from them. Likewise, private companies had stopped investing in ostrich farming. Large farms disappeared with discontinuation of breeders acquisition and distribution programs, as their business models focused heavily on the sale of breeding stock rather than the development and sale of finished products.

The event that buried the commercial ostrich farming occurred in 2012, when the farm with Federal Inspection Type certification was frozen for alleged involvement in organized crime. This was the last big ostrich production

farm that existed in Mexico. Its legacy was that it was a genetic source for most of the companies currently producing ostriches in Mexico, including one that had Federal Inspection Type certificate by 2016.

From 1988 to the present, SEMARNAT has a record of 320 commercial ostrich farms in Mexico. According to COMEPA data, the number of farms that had included ostriches in their livestock supply, but considering all non-officially registered farms, was close to 800. The latest COMEPA data referred to the year 2012 and reveal that 845 tons of ostrich meat was sold in Mexico that year, which was approximately 24,000 processed birds. This product was concentrated by a small number of producers located in the states of Coahuila, Zacatecas, Nayarit, Michoacán, Querétaro, Puebla, Tlaxcala, Guanajuato, Jalisco, Morelos, and the State of Mexico.

Current farms

The ostrich in Mexico is a very small and specialized sector of animal production. According to the current and former farmers, former FIRA officials and the leaders of the now-extinct producer organizations, were around 30 farmers who are still active in the market. In summer 2016, the present study identified 22 farms, of which 20 had agreed to provide information. Farms are located in the states of Tlaxcala (2 farms), State of Mexico (4 farms), Querétaro (1 farm), Guanajuato (3 farms), Michoacán (2 farms), Jalisco (3 farms), Nayarit (2 farms), Zacatecas (1 farm), Coahuila (1 farm) and Chihuahua (1 farm).

Table 1indicates the profile of Mexican farms that on 2016 produced ostrich and incorporated their products into the market. On average, the farms were small because they had a flock with around 30 breeders, which was in contrast to the size of the companies that existed in Mexico a few decades ago. On average, the farms were around 15 years old, although some were more than 20 years old and others that were created recently in 2016. The farms were in temperate and slightly elevated areas and produced predominantly on their land. The third part also produced ostriches and other species such as sheep and goats as well as other birds such as turkeys, ducks, emus and pheasants. More than half of farmers had higher education, and for more than half of them represented the ostrich as their main source of income. Another aspect that attracted attention was that most farmers had no experience in animal husbandry. Finally, it should be noted that the link was a property that existed on farms and their owners, as about half of them had contact with universities, media, and ostrich farms outside of Mexico.

Table 1. Profile of ostrich farms in Mexico in 2016

Characteristic	Value (n=20)
Farm age (years)	11.5 ± 6.4
Altitude of the territories (masl)	1740 ± 687
Temperature of the territories (°C)	18.9 ± 3.9
Flock size (birds)	32 ± 28
Farms that produce other species (%)	35
Farms that produce on their land (%)	75
Farms that interact with universities (%)	60
Farms that have had contact with the media (%)	40
Farms that maintain contact with a farm abroad (%)	50
Farms with owners with higher education (%)	60
Farms that represent the main source of income for their owners (%)	55
Farms with owners with livestock experience (%)	40

Source: Elaboration with field information 2016. *masl = meters above sea level.

The technical profile and productivity of current Mexican ostrich farms are described in Table 2. The description was organized considering three phases of the production process including reproduction, incubation and birth, as well as breeding and fattening.

Concerning the breeding stage, half of the farms produced their breeding stock and the other half acquired it. The breeders had an average age of around seven years in 2016. The productive unit is mainly used by trios consisting of two females and one male. The most of farmers changed the pen of breeders to achieve better productivity. The pens, in which each breeder's trio was housed were about 450 square meters, so each breeder had about a third of this space. The most farmers feed their breeders with specialty food in this stage of production. In terms of husbandry, all farmers immediately collected the eggs, managed to collect an average of 50 eggs per female, the vast majority of them was disinfected, and it took an average of one week for the eggs to be introduced into the incubator.

In terms of incubation and birth, all of the farms had an incubator, although the capacity of these devices varied greatly, ranging from 36 to 480 eggs. The incubation condition was homogenous with an average at 36 ° C and with 24% relative humidity. The incubators perform the flips automatically, with the number of flips per day programmed by the owners between one and four. Ovoscopy was performed on all farms, mainly on the 21st incubation day, but only one-third of the farmers had an electronic ovoscope. If the owners considered it appropriate, they moved the eggs to the hatchery, some days before day 40, others later. Therefore, the number of days that the chicks spent in the hatchery varied and on

average they remained three days. In most cases, chicks were assisted at birth and they received navel disinfection with iodine solutions. The chicks that could be born received food in the third day on average. Up to this step approximately 30 chicks per female were obtained.

In the breeding and fattening phase, most of farmers separated the chicks by age for their development and fattening. During this phase, less than half had access to veterinary services and ostrich production specialists. Due to the low commercial management of the species in Mexico, most of farmers had fattened their ostriches with other species´ food or with mixtures formulate by them. Only about 18 ostriches per female had reached the weight and slaughter age of 110 kg and 13 months on average. There were a few farms which the birds were slaughtered within their facilities under backyard conditions. On average, 63 kg of channel were obtained by ostrich (57% yield based on the weight of the live animal) and 33 kg of meat extracted (30% yield based on live weight).

Table 2. Technical profile and productivity of ostrich farms in Mexico in 2016.

Stage	Characteristic	Value (n=20)
Reproduction	Farms with purchased breeders (%)	50
	Age of the breeders (years)	6.7 ± 2
	Farms that form breeding triplets - two females and one male - (%)	80
	Area allocated by breeder (m2)	156 ± 133
	Farms that perform breeder rotation (%)	65
	Farms that supply specialized food for reproduction (%)	80
	Farms that perform egg disinfection (%)	95
	Storage of eggs prior to incubation (days)	7 ± 3.5
	Eggs obtained per female per year	50 ± 19
Incubation and birth	Farms that have their incubator (%)	100
	Incubator capacity (eggs)	158 ± 126
	Incubation temperature (°C)	36.2 ± 0.8
	Relative humidity in incubation (%)	24.3 ± 5.3
	Rotation during incubation (flips / day)	16 ± 10
	Farms that have an electronic ovoscope (%)	35
	Stay in the hatchery (days)	3 ± 2
	Farms assisting the birth of their chicks (%)	65
	Farms that perform navel disinfection (%)	70
	Start of feeding (days)	3 ± 2
	Chicks obtained per female per year	31 ± 18
Breeding and fattening	Farms that separate the chicks by age during their breeding and development (%)	90
	Farms that had access to specialized production consultancy (%)	40
	Farms with veterinary service (%)	40
	Farms that gain weight with specialized ostrich food (%)	15
	Birds achieved per female per year	18 ± 8
	Age of sacrifice (months)	13 ± 2.5
	Weight reached at slaughter (kg)	110 ± 9
	Farms that sacrifice in backyard conditions (%)	40
	Channel Weight (kg)	63 ± 11.5
	Yield in channel (%)	57 ± 7.5
	Meat obtained per bird (kg)	33 ± 7
	Yield in meat (%)	30 ± 6

Source: Elaboration with field information 2016.

Table **3** presents the commercial profile of ostrich producing mexican farms that were active in 2016. As demonstrated, ostrich farmers in Mexico generated revenue from sales of meat, skin, leather, leather goods, standing ostriches, eggs, shells, feathers and fat. In general, the sale of meat and live birds generated most of the income. However, some farms had obtained attractive benefits from selling finished leather goods and ostrich-based cosmetics. There were big differences in the prices

with which the farms could market the different products, and also in the weights that each product had in the composition of the income of the farms.

Regarding the added value, half of the farms had their meat processing facilities and obtained special cuts. However, only three offered meat in presentations of less than one-kilogram content, only two farms had an official safety certificate and only one of them sold meat by self-service stores. The favorite message for promoting meat was that it was a source of animal protein with excellent nutritional qualities. Tanning, the manufacture of leather goods and the manufacture of cosmetics were tasks outsourced to other companies, to which farmers supplied raw materials and were returned standardized end products. Just less than half of the farms had their brand through which they marketed their products, and only a one-third repeatedly exported some of their products. The most important advertising media were digital social networks.

Table 3. Products and activities for adding value to ostrich farms in Mexico in 2016

Characteristic	Value (n = 20)
Price per kilogram of meat (US $)	10 ± 4
Price per skin (US $)	84 ± 29
Price for leather (US $)	302 ± 230
Price per live animal sold (US $)	176 ± 93
Price per egg sold (US $)	9 ± 4
Price per shell sold (US $)	5 ± 3
Percentage of income from meat sales	37 ± 27
Percentage of income from skin sales	5 ± 7
Percentage of income from sales of leather and leather goods	14 ± 22
Percentage of income from sales of live birds	32 ± 32
Percentage of income from egg sales	4 ± 8
Percentage of income from the sale of pens	1 ± 2
Percentage of income from fat sales	4 ± 8
Percentage of income from shell sales	2 ± 2
Farms with meat processing facilities (%)	50
Farms that sell meat in specific cuts (%)	55
Farms that sell meat in presentations smaller than one kilogram (%)	15
Farms with meat safety certification (%)	10
Farms that sell their products in self-service (%)	5
Farms that highlight the nutritional qualities of ostrich meat in their promotional work	75
Farms that perform tanning of the skins (%)	5
Farms that manufacture leather goods (%)	0
Farms that have a brand for their products (%)	40
Farms that promote their products through social networks (%)	45
Farms that have exported a product (%)	30

Source: Elaboration with field information 2016.

DISCUSSION

Mexico had adequate climatic conditions for the commercial production of ostrich, labor and food were cheaper compared to other producer countries and for a whole decade (1998-2008) the activity was strongly promoted by different instances of the public sector. These circumstances made Mexico one of the countries with the greatest potential for commercial ostrich production (Carbajo, 2006). However, 30 years later, the sector collapsed and only a small number of small-scale individual producers remained.

In the words of the interviewees of this study, the large ostrich producers that existed in Mexico in recent years did not survive because their business depended heavily on the sale of breeding birds, which were massively marketed at high prices during the Mexican heyday. As the number of people interested in entering to an activity decreased, the price of the breeding stock collapsed and the decline began since productivity was low and the preparation and sale of value-added products in the business model was not yet consolidated the companies. In Kuwait and Greece ostrich production had a similar fate, the big farms concentrated on increasing the

number of birds and not on the sale of meat. In addition, they lacked adequate knowledge of management and nutrition, and the activity ended up was done by small individual producers (Theodoropoulou et al., 2001; Al-Nasser et al., 2003).

For their part, small farmers in Mexico faced various problems in both production and marketing which prevented them from succeeding. Low incubation efficiency and high mortality were the main problems on the production side. The lack of knowledge about incubation, nutritional formulation and disease management was the reason for the low productive efficiency of the farms. Similar reasons had been reported in countries such as Botswana (Moreki et al., 2012), Colombia (Mariño-González et al., 2017), Kazakhstan (Shameyeva et al., 2018) and Pakistan (Abbas et al., 2018). The lack of knowledge for the proper management of the ostrich could be resolved with competent extension services that enabled ostrich producers to develop their activities on a scientific basis (Abbas et al., 2018). In Mexico, however, there were only a few professionals specializing in the management of this specie. Indeed, it was known that the Mexican farms that remained in the business did not obtain their knowledge from institutional sources, but by experimenting and interacting with their national and international counterparts (Islas-Moreno and Rendón-Medel, 2019).

On the commercial side, Mexican small farmers encountered problems in the development and sale of value-added products, as there was a lack of infrastructure and because in general, ostrich products were little known in Mexico. This despite the evidence that exists on the qualities of the main products including meat, skin, oil, and feathers. In Botswana, the activity collapsed mainly due to the lack of infrastructure for the slaughter and processing of ostriches (Moreki et al., 2012), and in Pakistan the promotion of ostrich meat national consumption was recognized as an important task that those involved should include on their agenda (Abbas et al., 2018). The failure to find a demand for ostrich products was the main reason for the failure of commercial production of this species in many countries where it was incorporated (Benson, 2012). Future demand, which was mainly for meat, was overestimated, because it was assumed that ostrich meat would replace beef after the outbreaks of Spongiform Encephalopathy in 1986 and 2003 in Europe and the United States, respectively. However, such a substitution was never made.

Similar weaknesses in the ostrich farms professionalization, infrastructure, regulation and market had been identified in recent studies in Nigeria (Buochuama, 2018) and Pakistan (Abbas et al., 2018). On the other hand, the countries where commercial ostrich production was developing successfully had something in common, the participants were well organized. South Africa, the world leader in the production and export of ostrich products, was the best example, which had a national business chamber of commerce consisting of a farmer organization, a processing organization and two major export cooperatives focused on commercialization (Mabaya et al., 2011). An example of the strength of the South African structure of the sector was that it had succeeded in reestablishing itself thanks to the biosecurity measures carried out by all participants following the outbreak of H5N2 avian influenza, which in 2011 caused the loss of 10% of ostrich population in the country (Van Helden et al., 2016).

Zimbabwe was another country where ostrich production was deeply rooted. For the ostrich farmers of this country, associativity allowed them to had slaughterhouses and tanneries to guarantee the strict export controls, and thus had a 15 years prosperity period (1985-2000). However, the sector had experienced a sharp decline due to the agrarian reform, which would lead to a decline in agriculture and hyperinflation in 2000, reaching its most critical point in 2008. As a result, the inputs reached prohibitive prices and generated an environment of great uncertainty among investors (Cooper, 2007).

Poland was another country that successfully developed commercial exploitation of ostriches. Its success was due to its admission into the European Union, and the ability of its sector to organize and establish certified farms for the export of meat. 95% of the meat was exported to Western Europe, where ostrich meat was considered a good quality product that complements the meat offering. At the same time, ostrich farmers in Poland were taking advantage of their land, food and labor costs, and they had found a way to generate additional income in agritourism (Horbańczuk et al., 2008).

In Mexico, as in many other countries, commercial ostrich production had experienced an ephemeral heyday, based mainly on expectations and not on the realities of market demand. In addition, the rapid expansion of supply did not allow the natural development of processes for the production and dissemination of knowledge on the commercial management of the specie. As a result, there were large and small farmers who were productively inefficient and had great difficulty in developing, standardizing and marketing their products. The farms that remained in the business, continued with great difficulties

in terms of their productivity. However, they had managed to mitigate some of the problem by exchanging production practices and using digital media to consult their counterparts overseas. On the commercial side, they had managed to develop standardized products using maquiladora companies and placed them in niche markets where prices were higher than for conventional substitutes.

CONCLUSION

The high productive quality of a specie, the recognized attributes of its products and the great interest of different entities in participating in their use are not sufficient conditions for their economic success. In the case of ostrich, in Mexico and many other countries, the sector failed because market demand was overestimated and activity was started without adequate knowledge bases, infrastructure, and institutional support. Nevertheless, the surviving farms demonstrated that it was possible to stay in the activity by acquiring knowledge from interaction with other farms and developing standardized products for niche markets. Theses findings can refer to many other species of nascent interest.

DECLARATIONS

Acknowledgments

The sincerest gratitude is expressed to the National Council of Science and Technology (CONACYT), the body in charge of promoting science and technology in Mexico, for the financing granted to carry out the fieldwork of this work, as well as for providing the necessary support to finalize the postgraduate studies for two years.

Competing interests

The authors declare that they have no competing interests

Author´s contribution

Both authors contributed equally to the manuscript.

Consent to publish

All authors informed their consent prior to inclusion in the study

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JWPR
Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(4): 233-239, December 25, 2019

Research Paper, PII: S2322455X1900030-9
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DOI: https://dx.doi.org/10.36380/jwpr.2019.29

The Effects of Mospilan and Aktara Insecticides in the Feed on Egg Production and Meat Quality of Laying Hens

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Received: 30 Oct. 2019
Accepted: 08 Dec. 2019

ABSTRACT

The current study was aimed to investigate the effects of feeding Mospilan and Actara insecticides on egg production performance and meat quality of laying hens. Experimental research was conducted in the laboratory of the Department of Pharmacology and Toxicology of the National University of Life and Environmental Sciences of Ukraine in 2015. The experiments were performed on five groups each consisting of seven chickens. The age of the chickens at the beginning of the experiment was 150 days. The birds were fed the granulated compound feed. In M1 and M2 groups, Mospilan at doses of 65 mg/kg and 32.5 mg/kg of body weight were added to the feed, respectively. In A1 and A2 groups, Actara at doses of 360 mg/kg and 180 mg/kg of body weight were added to the feed, respectively. Chickens of the control group were fed without the addition of insecticides to the feed. The feeding period lasted 30 days and finally, egg production performance, meat quality, and gross pathological changes were evaluated. Egg production rate in M1 and M2 groups in comparison to the control group decreased by 78.4 and 29.7%, respectively. Egg production rate in A1 and A2 groups reduced by 89.2% and 48.7% compared to the control group, respectively. Chickens in groups of receiving insecticides had pale skin and enlarged heart, also showed spot hemorrhages in mucous membranes of the glandular stomach and intestine, color heterogeneity of the lungs, and the liver was dark cherry in color with hemorrhage. In addition, the relative weights of internal organs decreased by 23-36% in experimental groups. In the experimental groups, the pH of meat decreased at day 4 post-slaughter, and the meat broth with the addition of 5% copper sulfate solution was slightly cloudy with flakes. The meat of birds from the experimental groups was low toxic. Extracts from chicken meat of the experimental groups caused pathological changes, inhibition of movements and death of 13-16% of *Tetrahymena pyriformis* infusoria. This study demonstrated that the presence of Mospilan and Aktara in feed reduced the egg production rate, caused chronic poisoning, changed biochemical processes in chicken meat and increased its toxicity.

Key words: Chicken meat quality, Egg productivity, Insecticides Mospilan and Actara, Laying hens, Neonicotinoids.

INTRODUCTION

The use of chemical plant protection products, including insecticides, is an integral component of modern agricultural production. Until the year 2000, organo-phosphorus, pyrethroids and carbamates comprised 80% of the global production of insecticides. Nowadays, new generation compounds such as neonicotinoids, are widely used and already registered in almost 100 countries (Kovalenko et al., 2010). In Ukraine, more than 150 insecticides on the basis of five neonicotinoid active substances, namely imidacloprid, thiacloprid, thiameth-oxam, acetamiprid, and clothianidin are approved. Today, the most widely used neonicotinoids are thiamethoxam and acetamiprid (Kovalenko et al., 2010; Sekun, 2012; Govorov et al., 2013) and the commercial insecticides of Actara and Mospilan have been developed upon their basis, respectively. In Ukraine, these drugs according to "Hygienic classification of pesticides by degree of danger" (MHU, 1998) belong to Class IV toxicity (low toxic substances; LD_{50} = 501-5000 mg/kg of body weight in mice) and have high efficacy, low accumulation in mammalian tissues, and moderate persistence in environment (Kovalenko et al., 2010; Sekun, 2012; Govorov et al., 2013; Lin et al., 2019).

The intensive use of pesticides in crop production leads to the accumulation in animal and poultry feed

(Tomizawa et al., 2009). Moreover, farmers may apply pesticides in violation of technical guidelines (using excessive concentrations, increasing the frequency of application, ignoring the required preharvest intervals), which contributes to the accumulation of pesticide residues in the environment (Bartlett et al., 2019) and animal tissues, which negatively affect human health (Craddock et al., 2019). In this regard, cases of neonicotinoids poisoning in animals and birds have increased in recent years (See, 2009; Seceroglu et al., 2012; Lopez-Antia, 2015). In addition, neonicotinoids have been detected in foods of animal origin such as milk, meat and eggs (Seccia et al., 2008; Selvi et al., 2012; Yang et al., 2012; Lachat et al., 2018).

There are almost no data on toxicity and long-term impacts of accumulation of acetamiprid and thiamethoxam in the organs and tissues of animals and birds. Therefore, the investigations are needed to address the potential risks of neonicotinoids to facilitate their safe use in crop production. The aim of the present study was to evaluate the effects of the long-term intake of Mospilan and Actara insecticides on egg production, quality, and toxicity of chicken meat as well as gross pathological aspects in internal organs.

MATERIALS AND METHODS

Ethical approval

All animal experiments were in accordance with the Law of Ukraine «On the Protection of Animals from Brutal Treatment» and the recommendations of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Animals and study design

This study was conducted in the laboratory of the Department of Pharmacology and Toxicology of the National University of Life and Environmental Sciences of Ukraine in 2015. The experiments were performed on 35 laying hens, Belarus-9 cross, with a mean body weight of 1049 ± 50 g. The age of the chickens at the beginning of the experiment was 150 days. The birds were kept in the vivarium in accordance with the Sanitary Rules on the Organization, Equipment, and Maintenance of Experimental-Biological Clinics (Vivarium) in Ukraine. The hens were placed in five cages (1×1 m^2) with seven chickens in each cage. Each cage was equipped with a 40 W bulb. The chickens were fed the granulated compound commercial feed (Kalinka-7021, Trouw Nutrition Ukraine). The compound feed consisted of corn (30%), wheat (20%), soybean meal (20%), sunflower meal (15.5%), soybean oil (1.5%), enzymes, limestone, kitchen salt, monocalcium phosphate, vitamin, and mineral premix. The nutrient composition of the feed is presented in table 1.

Table 1. Nutrient composition of feed for laying hens

Nutrient	Value
Crude fiber (%)	4.2
Crude fat (%)	3.6
Calcium (%)	3.8
Phosphorus (%)	0.59
Energy, Kcal/100 g	305
Total protein (%)	14

Feed was given on average 100 g /chicken/day and water provided *ad libitum*. Before the beginning of the experiment, the birds were kept for 15 days as an adaptation period. The experiment included five groups with seven birds in each group. Chickens in the control group were fed the basal diet without additives. The birds in M1 and M2 groups were fed the basal diet containing the Mospilan (Nippon Soda Co., Ltd, Japan) at a dose of 65 and 32.5 mg/kg of average body weight, respectively (equal to 1/10 and 1/20 LD_{50} in mice, respectively). The chickens in A1 and A2 groups were fed the basal diet containing the Actara (Kwizda Agro GmbH, Austria) at a dose of 360 and 180 mg/kg of average body weight, respectively (equal to 1/10 and 1/20 LD_{50} in mice, respectively).

The experiment period lasted 30 days. The birds were monitored throughout the experiment. Consideration was given to appearance, reaction to external stimuli, the intensity and nature of the locomotor activity, the condition of the feather cover and mucous membranes. Also, the birds were observed for changes in body position, behavior, feed and water intake. The egg production of laying hens was determined by counting the number of eggs laid per 10 days in each group. At the end of the experiment, chickens were slaughtered to examine pathological changes and to evaluate the meat quality.

Laboratory studies

Pre-slaughter inspection and post-mortem examinations were carried out in accordance with Ukraine "Rules of veterinary inspection of slaughtered animals and veterinary examination of meat and meat products" (MAPU, 2002, Yakubchak, 2012). Pathological changes were evaluated by gross examination of the internal

organs. In addition, relative weights of internal organs (liver, spleen, lungs, heart, muscular and glandular stomach) calculated by the following formula:

$$RW = \frac{\text{organ weight (g)}}{\text{body weight (g)}} x100\%$$

Meat quality was evaluated according to (URTCSCQ, 2015). Muscle tissue samples were collected from each slaughtered bird. The freshness of the meat was determined 24 hours and 4 days post-slaughter. To assess the meat freshness by the reaction of meat broth with a 5% solution of copper sulfate, 20 g of shredded meat and 60 ml of distilled water placed in a conical flask. The flask was placed in a boiling water bath for 10 minutes and after that, the broth was filtered. Then 2 ml filtered broth was poured into a test tube and 3 drops of 5% copper sulfate solution was added, the test tube was shaken and left for 5 minutes, then results from reaction read.

In addition, the freshness of meat was evaluated by reaction with peroxidase. For this, 2 ml of water extract of meat was poured into a test tube and 5 drops of 0.2% alcoholic solution of benzidine and 2 drops of 1% hydrogen peroxide solution added.

The pH of meat determined by the potentiometric method of URTCSCQ (2015) in aqueous extract

The toxicity of the chicken meat was determined using the *Tetrahymena pyriformis* infusorium as a test organism (Lemesh et all 1997). Briefly, 50 mg of muscle tissue from each meat sample and 8 ml of 0.56% solution of pharmacy sea salt were poured into a porcelain mortar, then mixed to make a homogeneous mass. Next, 2 ml of homogeneous mass was put in a glass vial and one drop of a three-day culture of *Tetrahymena pyriformis* infusorium strain WH14, grown on peptone medium, added. The vials were closed with corks with holes, shacked and placed in a laboratory container and incubated at 25 °C for 3 days. After incubation, from each vial, one drop of infusoria culture was placed on a slide and examined under a microscope with low magnification. Toxicity was assessed by the presence of dead infusoria, inhibition of growth as well as changes in their shape and movement.

Statistical analysis

The results were statistically processed by using the Microsoft Excel Data Analysis ToolPak. The differences between the values were evaluated using the Student's t-test. A p-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

During the experiment, chickens in the control and experimental groups moved actively and responded appropriately to external stimuli. Body temperature was within the physiological range (41-42 °C). Visible clinical signs of poisoning and death were not found.

Egg production performance

Egg production in M1 group in comparison to control group decreased by 9.7, 51.4 and 78.4% during the first, second and third 10 days of study, respectively ($p \leq 0.05$), while reduction in egg production in M2 group was 2, 8.6 and 29.7% in aforementioned intervals, respectively ($p \leq 0.05$) (Figure 1). Egg production in A1 group recorded 6.5, 68.6 and 89.2% reduction during the first, second and third 10 days of the experimental period, which was significantly different compared to the control group ($p \leq 0.05$). The egg production rate of A2 group was similar to that of the control group during the first 20 days of study. However, during 21 to 30 days of study, the production rate in A2 group in comparison to the control group decreased by 48.7% ($p \leq 0.05$) (Figure 1).

The decrease in egg production indicates metabolic disorders, the development of profound changes in internal organs and the body as a whole (Bazaka et al., 2017). In this study, the reducing effect of Mospilan on the egg production of chickens was less than the Actara effect, although the toxicity of Mospilan was significantly higher for mice in the laboratory experiments (Bazaka et al., 2017). It is known that chronic pesticide poisoning disrupts the barrier function of the intestinal mucosa, thereby providing conditions for the spread of intestinal microflora in the body and the occurrence of secondary infections (See, 2009; Seceroglu et al., 2012; Lopez-Antia, 2015).

According to Millot et al. (2017) large-scale use of neonicotinoid insecticides has raised growing concerns about their potential adverse effects on farmland birds. They reviewed the mortality incidents partridges and pigeons, for which toxicological analyses detected imidacloprid residues. Mortality was due to poisoning by imidacloprid-treated seeds.

According to Van Lexmond et al. (2015) consumption of small numbers of dressed seeds offers a potential route for direct mortality in granivorous mammals and birds, for such birds need to eat only a few spilt seeds to receive a lethal dose. Lower doses lead to a range of symptoms including impaired immune function, reduced fecundity and lethargy.

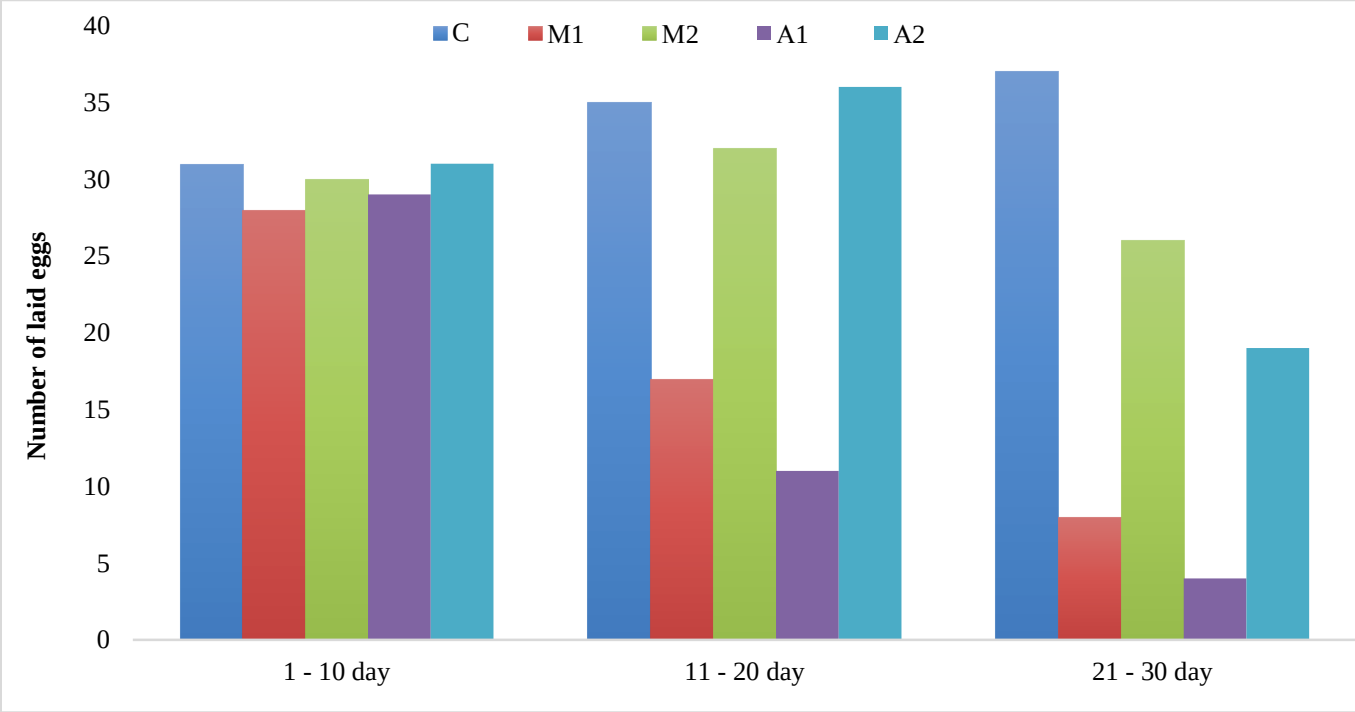

Figure 1. Egg production performance of laying hens in presence of Mospilan and Aktara insecticides in the feed. M1 and M2 groups were fed the basal diet containing the Mospilan at a dose of 65 and 32.5 mg/kg of body weight, respectively. A1 and A2 groups were fed the basal diet containing the Actara at a dose of 360 and 180 mg/kg of body weight, respectively. C: Control group.

Meat quality

Inspection of carcasses of birds slaughtered in experimental groups (M1, M2, A1, and A2) revealed that the surfaces of carcasses were pale and had a distinct smell, which was not typical for fresh poultry. Other organoleptic parameters corresponded to fresh meat.On day 4 post-slaughter, pH values of meat in M2 and A1 groups were less than that in M1, A2 and control groups; whereas the pH of chicken meat in experimental groups at 1 day post-slaughter was slightly different from that of the control group (Figure 2).

Assessment of the meat quality by the reaction of broth with 5% copper sulfate solution indicated that the meat broth in samples obtained from the control group was clear. While the broth samples from the experimental groups were cloudy with flakes appearance, indicating the occurrence of biochemical changes in the meat and doubtful freshness of chicken meat.

Determination of peroxidase activity in meat samples from control and experimental groups after 1 and 4 days storage at 2-4 °C showed that aqueous extracts were blue-green, which turned brown. This indicated a high activity of peroxidase.

Thus, the results of the veterinary-sanitary evaluation of laying carcasses indicated some disturbances of biochemical processes in tissues affected by Mospilan and Actara that may have negative effects on the quality and safety of meat over long periods of storage.

The evaluation of toxicity using the *Tetrahymena pyriformis* infusorium revealed that chicken meat of all experimental groups was slightly toxic. The meat extracts of chickens in the M1 group resulted in the death of 18% of *Tetrahymena pyriformis*, inhibition of movements in 76% infusoria and morphological changes in 2.0% infusoria. Extracts of poultry meat obtained from the M2 group resulted in the death of 16.0% of infusoria, inhibition of movements in 79.0% infusoria and morphological changes in 1% cases.

Chicken meat extracts of the A1 group led to the death of 15.0% of infusoria, also cells with inhibited movements and morphological changes were 75% and 4%, respectively. The results of the toxicological evaluation of the chicken meat in the A2 group revealed that 13% of infusions were dead, 75% and 4.0% were cells with inhibited movements and morphological changes, respectively.

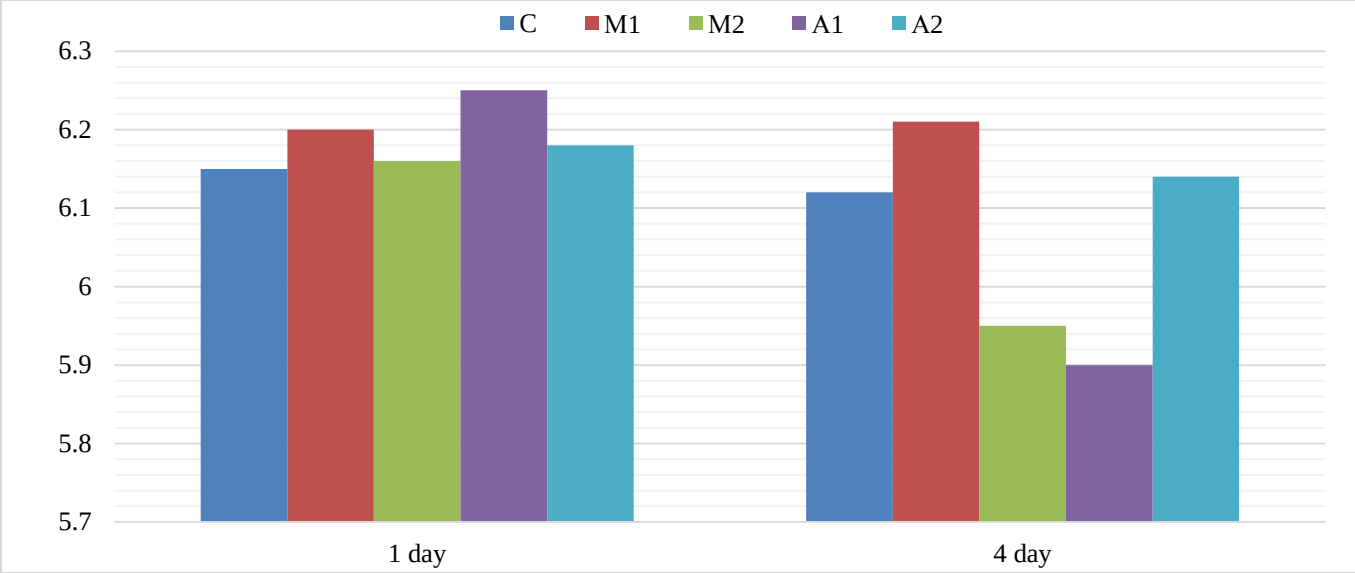

Figure 2. The pH of meat of laying hens in presence of Mospilan and Aktara insecticides in the feed. M1 and M2 groups were fed the basal diet containing the Mospilan at a dose of 65 and 32.5 mg/kg of body weight, respectively. A1 and A2 groups were fed the basal diet containing the Actara at a dose of 360 and 180 mg/kg of body weight, respectively. C: Control group

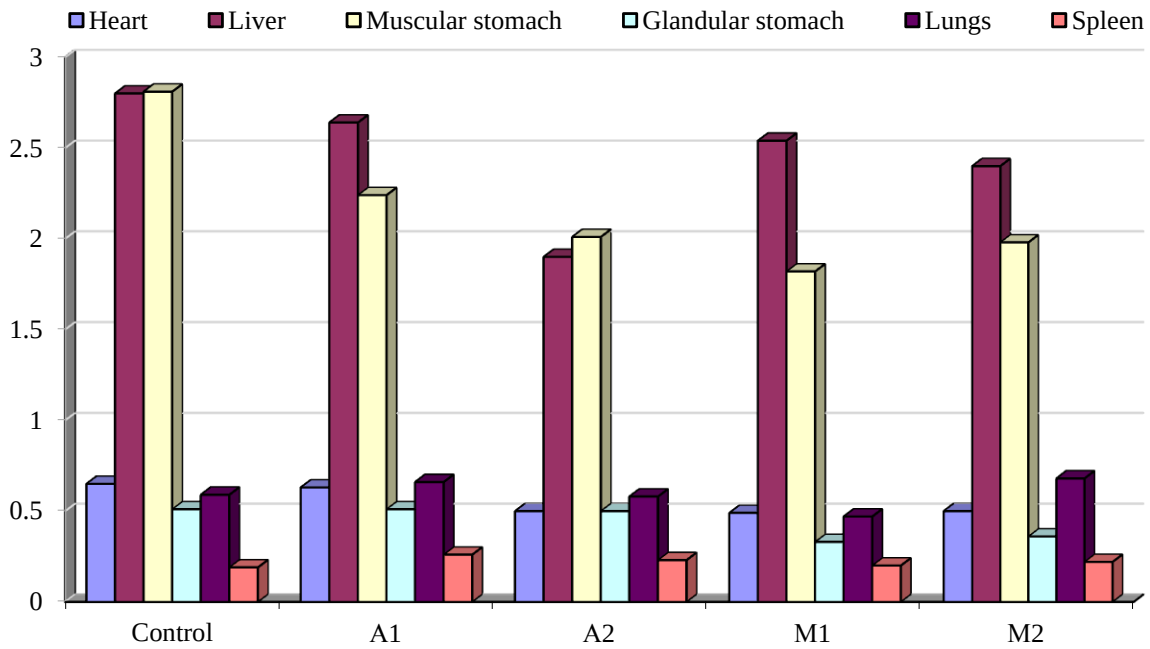

Figure 3. The relative weights of the internal organs of laying hens in presence of Mospilan and Aktara insecticides in the feed. M1 and M2 groups were fed the basal diet containing the Mospilan at a dose of 65 and 32.5 mg/kg of body weight, respectively. A1 and A2 groups were fed the basal diet containing the Actara at a dose of 360 and 180 mg/kg of body weight, respectively. C: Control group.

The relative weights of the internal organs are shown in figure 3. The obtained results in M1 group indicated a significant decrease in the relative weight of heart by 25%, liver by 9%, lungs by 20%, and glandular stomach by 35% compared to the control group ($p \leq 0.05$). In A1 group in comparison to the control, a decrease in the relative weight of liver, lungs, and muscular stomach, as well as an increase in the relative weight of spleen by 37% was observed ($p \leq 0.05$). In A2 group, the relative weight of heart significantly decreased by 23.0%, and liver by 32.0%, however, the relative spleen weight was 21% higher than that in the control group ($p \leq 0.05$).

Gross examination of the internal organs of laying hens in the experimental groups revealed heterogeneity in color of lungs, enlargement of the heart, hemorrhage in the liver, spot hemorrhages on the mucous membrane of the glandular stomach and intestines. Also, some chickens showed swelling of the small intestine. The pathological changes in the body indicated chronic poisoning. Reduction of egg production, deterioration and pathomorphological changes in the body of birds demonstrated the negative effect of insecticides Mospilan and Actara, even at doses of 1/10 and 1/20 LD_{50} for mice.

CONCLUSION

The contamination of feed of laying hens with Mospilan and Actara in subtoxic doses caused chronic poisoning, which led to decreased egg production and meat quality, reduced the weight of the liver, kidneys, lungs, stomach as well as hemorrhages in the liver and mucous membrane of the glandular stomach and intestines.

DECLARATIONS

Author's contribution

Galina Bazaka and Volodymyr Dukhnytskyi conducted the research, collected data and performed the statistical analysis. Vasily Sokolyuk, Petro Boiko and Irina Ligomina wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors have declared that no competing interest exists.

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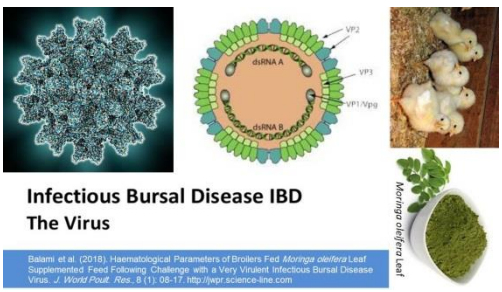

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AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

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

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

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

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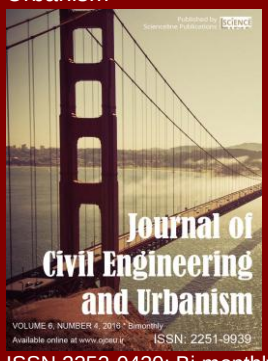

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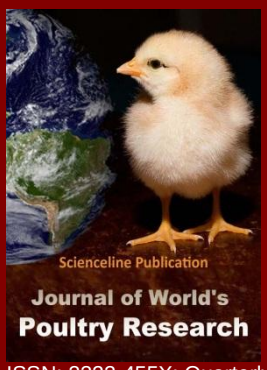

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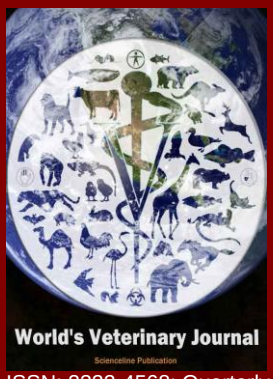

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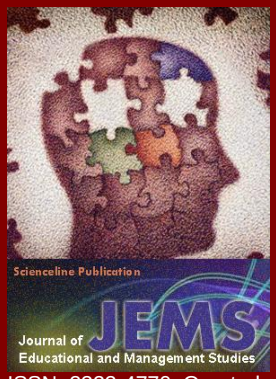

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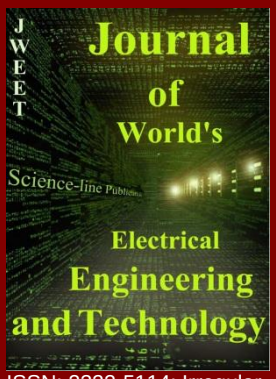

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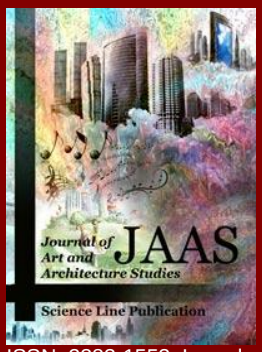

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